The Dissertation Committee for Catherine Rachel Darst

certifies that this is the approved version of the following dissertation:

Evolutionary and Ecological Dynamics of Aposematism and Mimicry in Poison Frogs

Committee:

_____
David C. Cannatella, Supervisor

_____
Molly E. Cummings, Supervisor

_____
Michael P. Domjan

_____
Ulrich G. Mueller

_____
Michael J. Ryan

Evolutionary and Ecological Dynamics of Aposematism and Mimicry in Poison Frogs

by

Catherine Rachel Darst, B.A.S.

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This dissertation is dedicated to all the

women who were ever told that they

succeeded only because of their gender.

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Evolutionary and Ecological Dynamics of Aposematism and Mimicry in Poison Frogs

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Catherine Rachel Darst, Ph.D.
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Supervisors: David C. Cannatella and Molly E. Cummings

In aposematism, defended prey advertise their aversive qualities to predators using a warning signal. Predators that have learned the warning signal are thus the selective agent that can promote similarity in warning signals between distantly related species (i.e. defensive mimicry). Here, I use poison frogs to study the evolutionary and ecological dynamics of aposematism and mimicry. An incredible diversity of color patterns exists among poison frogs and this bright coloration has evolved multiple times from cryptic ancestors, making them a unique system for investigating the complexities and controversies of warning signals.

In chapter 1, I explore the phylogenetic relationships of poison frogs (Dendrobatidae) to other frogs, testing the assumption that lack of bright coloration and alkaloid skin toxins is ancestral for dendrobatids. Most skin toxins of poison frogs are sequestered from their diet. In chapter 2, I examine a critical prediction of the diet-

toxicity hypothesis, which states that independent origins of dietary specialization will be correlated with independent origins of chemical defense. Using comparative methods, I found a recurring association of dietary specialization and alkaloid sequestration, suggesting parallel evolutionary trends in the origins of aposematism. In chapter 3, I investigate the relative importance of aposematic signal components, conspicuousness and unpalatability, for anti-predator defense using natural signal variation among poison frogs of Ecuadorian Amazonia. I found equally effective predator avoidance strategies with differential investment in conspicuous coloration and toxicity across species, demonstrating a mechanistic explanation for natural diversity in warning signals and providing empirical evidence for Batesian mimicry in dendrobatid frogs. In chapter 4, I examine the dynamics of Batesian mimicry where mutiple model species co-occur and, therefore, more than one warning signal could be exploited by the mimic. I demonstrate that mimics resemble the less toxic and less abundant model species, and that this counter-intuituve mimicry pattern is selectively advantageous due to the psychological phenomenon of toxicity dependent stimulus-generalization in educated predators. Finally, in chapter 5, I explore how evidence from experimental psychology can further improve our conceptualization of predator learning and memory and therefore enhance our predictions for mimicry dynamics.

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Introduction

The idea that conspicuous coloration of defended (often toxic) prey has adaptive significance dates back to Wallace (1867, 1889). He suggested that the conspicuous traits of defended prey function as a warning signal of unprofitability to potential predators. Warning signals were shortly thereafter formally termed aposematic by Poulton (1890), defined as "…an appearance which warns off enemies because it denotes something unpleasant or dangerous." More than a century later, the evolutionary and ecological dynamics of aposematism continue to be studied and debated, due in large part to the dynamic interactions of evolution, ecology, physiology, and behavior of predators and prey (Ruxton et al. 2004; Speed & Ruxton 2004; Mappes et al. 2005).

Poison frogs (family Dendrobatidae) display some of the most diverse warning signals in nature. Phylogenetic analyses indicate that an incredible variety of color combinations has arisen multiple times from cryptic ancestors in dendrobatid frogs (Santos et al. 2003; Vences et al. 2003). Thus, poison frogs are a unique system for investigating the selective forces behind the evolutionary dynamics of aposematism. I began by investigating the relationship of dendrobatids to other neobatrachian frogs. Even though the precise sister group of Dendrobatidae is not clear, none of the apparent close relatives are aposematic, allowing for the assumption that lack of bright coloration and alkaloid skin toxins is ancestral for poison frogs (Darst and Cannatella 2004).

The noxious alkaloids in the skin of poison frogs are accumulated from dietary sources: small, leaf-litter arthropods, particularly ants (Daly et al. 1994, 2000, 2002; Saporito et al. 2003; 2004). A critical prediction of this diet-toxicity hypothesis is that independent origins of dietary specialization will be correlated with independent origins of chemical defense. Using comparative methods, I tested this hypothesis and found a recurring association of dietary specialization and alkaloid sequestration, suggesting parallel evolutionary trends in the origins of aposematism (Darst et al. 2005). I next examined the relative importance of aposematic signal components, conspicuousness and unpalatability, for anti-predator defense using natural signal variation among poison frogs of Ecuadorian Amazonia. I found equally effective predator avoidance strategies with differential investment in conspicuous coloration and toxicity across species (Darst et al. 2006). These results suggest that decoupling conspicuousness and unpalatability is favored for effectively and efficiently avoiding predation, demonstrating a mechanistic explanation for natural diversity in warning signals.

Defensive mimicry, similarity in warning signals between unrelated species, was a first test of Darwin's theory of natural selection (Bates 1862; Wallace 1865). Mimicry is generally categorized into two forms, Müllerian and Batesian. Müllerian mimicry is convergent and mutualistic because both brightly colored species are defended and therefore share the costs incurred by naïve predators learning to avoid the shared warning signal (Müller 1879). Batesian mimicry, on the other hand, is essentially parasitic: an edible species co-opts the warning coloration of a defended species, and in doing so, degrades the effectiveness of the signal (Bates 1862). The intricacies of mimicry

dynamics remain surprisingly controversial, mostly due to the complexities of natural systems and because predictions lie on a multi-disciplinary interface (Mallet 2001; Darst 2006).

Species of poison frogs in the genus *Epipedobates* share bright color patterns with *Allobates* species. This shared, apparent warning coloration, however, is not due to common ancestry; *Epipedobates* and *Allobates* species are distantly related, both being more closely related to cryptic *Colostethus* than one another (Santos et al. 2003; Vences et al. 2003). Using spectral reflectance, toxicity assays, and predator learning experiments with live poison frogs and naïve avian predators, I found the non-toxic *Allobates* mimics successfully deceive predators trained with the toxic *Epipedobates* model species, providing the first experimental evidence for Batesian mimicry in frogs (Darst & Cummings 2006; Darst et al. 2006).

Batesian mimetic advantage is considered to be frequency-dependent because increased mimic abundance will lead to warning signal breakdown (Fischer 1930; Brower & Brower 1962). Thus, where multiple toxic model species are available, Batesian polymorphism is predicted—mimics diversify to match sympatric models. I found, however, that where two of the model *Epipedobates* species' ranges overlap, the *Allobates* mimics resemble only one of the models, the less toxic and less abundant species. Using predator learning and generalization experiments (Pavlov 1927; Duncan & Sheppard 1965), I found that predators differed in avoidance generalization depending on model toxicity, conferring greater protection to mimics resembling the less toxic

model (Darst & Cummings 2006). Because learned avoidance from experience with the more toxic model will generalize to either mimic phenotype, but learned avoidance from experience with the less toxic model will only generalize to the precise mimic, mimics of the less toxic model receive greater benefits, those generated by both models. Therefore, by mimicking the less toxic model (rather than mimetic polymorphism) the increased predation risk accrued by an increased abundance of Batesian mimic individuals is spread over both defended model species, revealing a monomorphic evolutionary solution for the problem of Batesian abundance.

To investigate the evolutionary and ecological dynamics of aposematism and mimicry, my dissertation research integrates phylogenetics (to understand evolutionary relationships), feeding ecology (to test the diet-toxicity hypothesis), chemical ecology (to determine relative toxicities), sensory ecology (to examine color pattern conspicuousness and discriminability), and behavioral ecology (to explore features of predator avoidance learning). My results provide insight into processes contributing to and maintaining warning coloration and mimicry, generate predictions for the evolutionary dynamics of aposematism, as well as lead to deeper understanding of the evolution of animal signals and receiver psychology.

The slight differences in formatting of the following five chapters result from minor differences in journal formatting. Chapter 1 has been previously published as Darst & Canntella (2004) in Molecular Phylogenetics and Evolution; chapter 2 has been previously published as Darst et al. (2005) in The American Naturalist; chapter 3 is in

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References

Bates, H.W. 1862 Contributions to an insect fauna of the Amazon valley. Lepidoptera: Heliconidae. Transactions of the Linnaean Society of London 23:495–566.

Brower, L. P. & Brower, J. V. Z. 1962. The relative abundance of model and mimic butterflies in natural populations of the *Battus philenor* mimicry complex. Ecology 43: 154–158.

Daly, J. W., Garraffo, H. W., Spande, T. F., Jaramillo, C., & Rand, A. S. 1994. Dietary source for skin alkaloids of poison frogs (Dendrobatidae)? Journal of Chemical Ecology 20:943–955.

Daly, J. W., Garraffo, H. M., Poonam, J., Spande, T., Snelling, R. R., Jaramillo, C., & Rand, A. S. 2000. Arthropod-frog connection: decahydroquinoline and pyrrolizidine alkaloids common to microsympatric myrmicine ants and dendrobatid frogs. Journal of Chemical Ecology 26:73–85.

Daly, J. W., Kaneko, T., Wilham, J., Garraffo, H. M., Spande, T. F., Espinosa, A., & Donnelly, M. A. 2002. Bioactive alkaloids of frog skin: combinatorial bioprospecting reveals that pumiliotoxins have an arthropod source. Proceedings of the National Academy of Sciences USA 99:13996–14001.

Darst, C. R. 2006. Predator learning, experimental psychology, and novel predictions for mimicry dynamics. Animal Behaviour, in press.

Darst, C. R., & Cannatella, D. C. 2004. Novel relationships among hyloid frogs inferred from 12S and 16S mitochondrial DNA sequences. Molecular Phylogenetics and Evolution 31: 462–475.

Darst, C. R., & Cummings, M. E. 2006. Predator learning favours mimicry of a less toxic model in poison frogs. Nature 404: 208–211.

Darst, C. R., Menéndez-Guerrero, P. A., Coloma, L. A., & Cannatella, D. C. 2005. Evolution of dietary specialization and chemical defense in poison frogs (Dendrobatidae): a comparative analysis. American Naturalist 165: 56–69.

Darst, C. R., Cummings, M. E., & Cannatella, D. C. 2006. A mechanism for diversity in warning signals: conspicuousness versus toxicity in poison frogs. Proceedings of the National Academy of Sciences USA, in press.

Duncan, C. J. & Sheppard, P. M. 1965. Sensory discrimination and its role in the evolution of Batesian mimicry. Behaviour 24: 269–282.

Fisher, R. A. 1930. *Genetical theory of natural selection* Oxford: Clarendon Press, Oxford.

Mallet, J. 2001. Mimicry: an interface between psychology and evolution. Proceedings of the National Academy of Sciences USA 98: 8928–8930.

Mappes, J., Marples, N., & Endler, J. A. 2005. The complex business of aposematism. Trends in Ecology and Evolution 20: 598–603.

Müller, F. 1879. *Ituna* and *Thyridia*: a remarkable case of mimicry in butterflies. Transactions of the Entomological Society of London 1879:xx–xxix.

Pavlov, I. P. 1927. *Conditioned reflexes* (translation, Anrep, G. V.) London: University Press.

Poulton, E. B. 1890. *The Colours of Animals: Their Meaning and Use Especially Considered in the Case of Insects*. London: Kegan Paul, Trench, Trubner, and Co.

Ruxton, G. D., Sherratt, T. N., & Speed, M. P. 2004. *Avoiding attack: the evolutionary ecology of crypsis, aposematism, and mimicry*. Oxford: Oxford University Press.

Santos J. C., Coloma L. A., & Cannatella D. C. 2003. Multiple recurring origins of aposematism and diet specialization in poison frogs. Proceedings of the National Academy of Sciences USA 100: 12792–12797.

Saporito, R. A., Donnelly, M. A., Hoffman, R. L., Garraffo, H. M., & Daly, J. W. 2003. A siphonotid millipede (*Rhinotus*) as the source of spiropyrrolizidine oximes of dendrobatid frogs. Journal of Chemical Ecology 29:2781–2786.

Saporito, R.A., Garraffo, H.M., Donnelly, M.A., Edwards, A.L., Longino, J.T., & Daly, J.W. 2004. Formicine ants: an arthropod source for the pumiliotoxin alkaloids of dendrobatid poison frogs. Proceedings of the National Academy of Sciences USA 101:8045–8050.

Speed, M. P, & Ruxton, G. D. 2005. Aposematism: what should our starting point be? Proceedings of the Royal Society of London B: 272: 431–438.

Vences M. J., Kosuch J., Boistel R., Haddad C. F., LaMarca E., Lötters S., & Veith M. 2003. Convergent evolution of aposematic coloration in Neotropical poison frogs: a molecular phylogenetic perspective. Organismal Diversity and Evolution 3: 215–226.

Wallace, A. R. 1865. On the phenomena of variation and geographical distribution as illustrated by the Papilionidae of the Malayan region. Transactions of the Linnean Society of London 25: 1-71.

Wallace, A. R. 1867. *Proceedings of the Entomological Society of London*, 4 March, pp IXXX–IXXXi.

Wallace A. R. 1889. *Darwinism—an exposition of the theory of natural selection with some applications*. London: Macmillian.

Chapter 1

Novel relationships among hyloid frogs inferred from 12S and 16S mitochondrial DNA sequences*

Abstract: Advanced frogs (Neobatrachia) are usually divided into two taxa, Ranoidea (the firmisternal frogs) and Hyloidea (all other neobatrachians). We investigated phylogenetic relationships among several groups of Hyloidea using 12S and 16S rRNA mitochondrial gene sequences and tested explicit relationships of certain problematic hyloid taxa using a sample of 93 neobatrachians. Parsimony, maximum likelihood and Bayesian inference methods suggest that both the Ranoidea and Hyloidea are well-supported monophyletic groups. We reject three hypotheses using parametric bootstrap simulation: 1) Dendrobatidae lies within the Ranoidea; 2) The group containing Hylidae, Pseudidae, and Centrolenidae is monophyletic; 3) *Brachycephalus* is part of Bufonidae.

*Significant portions of this chapter have been previously published as Darst & Cannatella, 2004. Molecular Phylogenetics and Evolution 31: 462–475.

1.1 INTRODUCTION

The frogs and toads (Anura) include more than 4,800 species in at least 26 families (Frost 1985, 2002). Frogs were partitioned into Archaeobatrachia ("primitive" frogs) and Neobatrachia ("advanced" frogs) by Reig (1958) based on the presence of free ribs and the type of vertebrae in the "primitive" frogs; this arrangement was followed by Tihen (1965) and Duellman (1975). Based on morphological data, Cannatella (1985) and Ford and Cannatella (1993) argued that archaeobatrachians were paraphyletic with respect to Neobatrachia. In contrast, analyses based on DNA sequence data have supported the monophyly of Archaeobatrachia (Hay et al. 1995). The monophyly of Neobatrachia, however, was strongly supported by both molecular and morphological datasets.

The separation of the Neobatrachia into two units, Bufonoidea (more correctly, Hyloidea [Dubois, 1983]) and Ranoidea, has been accepted by most investigators of anuran classification since the mid-1800's (Lynch 1973). The separation of hyloids and ranoids rests on morphological characters: shape of the vertebral centrum, pectoral girdle architecture, and conformation of thigh musculature (Lynch 1973; Ford and Cannatella 1993). Whereas morphological studies have suggested that hyloids are paraphyletic to ranoids (Kluge and Farris 1969; Lynch 1971, 1973; Ford, 1989), molecular analyses corroborate two monophyletic groups, Hyloidea and Ranoidea (Hay et al. 1995; Ruvinsky and Maxson 1996; Vences et al. 2000). However, the placement of some basal neobatrachian clades (Heleophrynidae, Myobatrachidae, Sooglossidae) remains

uncertain. Given this, we here associate the name Hyloidea with a less inclusive and more stable clade, specifically the most recent common ancestor of Eleutherodactylini, Bufonidae, Centrolenidae, Phyllomedusinae, Pelodryadinae, and Ceratophryinae. This definition of Hyloidea is node-based (de Queiroz and Gauthier 1992) and we elaborate upon our rationale in the Discussion.

Within this more restricted clade Hyloidea, we address the relationships of certain taxa whose placement has been disputed. First, most morphological studies have proposed that Dendrobatidae, the poison frogs, be placed within Ranoidea based on the fusion of the epicoracoid cartilages (firmisterny) of the pectoral girdle (Griffiths 1959; Duellman and Trueb 1986; Ford and Cannatella 1993; Ford 1993), whereas molecular analyses have placed Dendrobatidae within Hyloidea (Hay et al. 1995; Ruvinsky and Maxson 1996; Vences et al. 2000).

A second area of conflict is the relationships of the Hylidae, Pseudidae and Centrolenidae. Pseudidae and Centrolenidae have traditionally been grouped together with the Hylidae based solely on the presence of intercalary elements, which are supernumerary skeletal elements between the distal and next-to-distal elements of the fingers and toes (Lynch 1973; Duellman and Trueb 1986; Ford and Cannatella 1993). Molecular data, however, have placed Pseudidae sister to either Rhinodermatidae or Leptodactylidae (Hay et al. 1995; Ruvinsky and Maxson 1996).

Brachycephalidae is also problematic. *Brachycephalus* was thought to be most closely related to *Atelopus* (Bufonidae) based on pectoral girdle similarities (Noble 1931;

Griffiths 1959; Lynch 1973). Later McDiarmid (1971) placed *Brachycephalus* in its own family based mostly on lack of a Bidder's organ, which is otherwise found only in Bufonidae. Recently, however, Brachycephalidae has been suggested to have a close relationship to *Euparkerella* (Izecksohn 1971, 1988), a leptodactylid of the tribe Eleutherodactylini. None of these phylogenetic hypotheses have been explicitly tested.

To address the phylogenetic relationships and test explicit phylogenetic hypotheses among the smaller hyloid families, we analyzed a 2.4kb region spanning 12S and 16S rRNA mitochondrial genes and the intervening tRNA valine in 93 neobatrachian taxa. We address the following questions: (1) Is Dendrobatidae part of Ranoidea or Hyloidea? (2) Do Hylidae, Centrolenidae and Pseudidae form an exclusive clade? (3) What is the relationship of *Brachycephalus* to other hyloideans?

1.2 MATERIALS AND METHODS

Taxa. We used 79 sequences from the ingroup (hyloid families Bufonidae, Dendrobatidae, Centrolenidae, Hylidae, Leptodactylidae, Brachycephalidae and Pseudidae). The only families of hyloids not sampled were Rhinodermatidae (two species) and Allophrynidae (one species). Monophyly of the ingroup is based on published analyses (Ruvinsky and Maxson 1996) as well as our unpublished data. Outgroup taxa consist of 14 sequences from Myobatrachidae, Heleophrynidae, and Ranoidea (Ranidae, Microhylidae, Rhacophoridae, and Hyperoliidae). Forty new

sequences were added to taxa previously sequenced in the Cannatella lab (Basso and Cannatella in prep.) to diversify taxon sampling so that relationships within Hyloidea could be estimated more accurately (Table 1.2). The taxonomy generally follows Frost (2002) except that we retained the use of *Hylactophryne* (rather than *Eleutherodactylus*) and *Phrynomerus* (rather than *Phrynomantis*). Also, Eleutherodactylini is treated as a tribe rather than the subfamily Eleutherodactylinae (Frost 2002; Laurent 1986).

DNA amplification and sequencing. Genomic DNA was extracted from liver or muscle tissue using the Quiagen DNAeasy™ kit. The polymerase chain reaction (PCR) was used to independently amplify four overlapping DNA fragments spanning 2.4kb of 12S and 16S mitochondrial rRNA genes and the intervening tRNA gene for valine, which corresponds to positions 2185–4574 in the complete mitochondrial sequence of *Xenopus laevis* (GenBank Accession NC 001573, derived from M10217; provisional reference sequence). Combinations of primers MVZ59, tRNAphe, tRNAval, MVZ50, 12L1, 16SH, 12SM, 16SA, 16SC, and 16SD were used (Goebel et al. 1999; Table 1.1). Standard PCR conditions (Palumbi, 1996) were used with the following thermal cycle profile: 2 min at 94°C, followed by 35 cycles of: 94°C for 30 s, 46°C for 30 s, 72°C for 60 s. Annealing temperature and/or numbers of cycles were slightly modified as needed to improve the quality of the PCR product. This product was purified using the QIAquick Gel Extraction Kit™. Cycle sequencing reactions were completed with ABI Prism BigDye Terminator chemistry (Versions 2 and 3; Applied Biosystems). Sequencing was performed on an ABI 3100 PRISM™ sequencer with the following

conditions for 25 cycles: 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. (Applied Biosystems, Inc.).

Sequence Analysis. Contiguous sequences from eight completely overlapping fragments were constructed in Sequencher 4.1 (GeneCodes Corp.), and DNA sequences were aligned using Clustal X 1.8 under a variety of gap penalty weightings (Thompson et al. 1997). Using MacClade 4.0 (Maddison and Maddison 2000), manual alignment adjustments were made to minimize informative sites under the parsimony criterion. Secondary structure models from the Gutell lab website (www.rna.icmb.utexas.edu) were used to help make decisions about ambiguous regions. Regions of the alignment for which homology of the sites could not be inferred were excluded from analysis.

Parsimony analyses were performed with PAUP* 4.0b8 (Swofford 2000) using heuristic searches under parsimony (all characters weighted equally, gaps were not scored as characters) with TBR branch swapping, and 1,000 random addition sequence replicates. In order to obtain estimates of clade support, nonparametric bootstrapping was performed with heuristic searches of 1,000 replicate datasets and 50 random addition sequences per dataset (Felsenstein 1985).

For maximum likelihood analyses, a model of sequence evolution was estimated for the data set using MODELTEST (Posada and Crandall 1998). Parameters were estimated from the most parsimonious trees and fixed for further analysis. Three independent maximum likelihood heuristic searches were performed with PAUP* 4.0b8

using random starting trees (rather than random-taxon addition). TBR branch swapping was used to swap to completion.

Bayesian analyses under the model determined by MODELTEST were performed with a beta version of MrBayes3b4 (Huelsenbeck and Ronquist 2001) on Phylocluster, a NPACI Rocks cluster (www.rocksclusters.org) composed of one master node with eight slave nodes, each of which uses dual AMD 1533 MHz processors with 2 GB RAM. The Bayesian analysis uses Markov Chain Monte Carlo to estimate the target posterior probability distribution over tree topologies and evolutionary model parameters. Preliminary runs were performed to assess the appropriateness of the default Markov Chain proposal settings. For the final four independent runs, the gamma shape parameter and base frequency proposal distributions were changed to allow between a 20-50% acceptance rate and therefore sample the target distribution more effectively. The default values of four Markov chains and the "temperature" parameter value of 0.2 were used to help avoid entrapment in local topological optima and to traverse tree space more broadly. The default priors were assumed: a uniform prior for topology, a uniform distribution (0,1) for proportion of invariant sites, a uniform distribution (0.1, 50) for the alpha shape parameter, and a prior of exp(10) for branch lengths. A uniform dirichlet distribution (multinomial form of the beta distribution) was assumed for base frequencies and the rate matrix. The Markov chain length was 5,000,000 generations for two of the runs, 4,800,000 generations for a third, and 4,770,000 generations for the fourth. All chains were sampled every 100 generations. The first 5,000 samples were discarded as burn-in; this value was found to be appropriate and conservative by plotting the

likelihood and parameter values of the four runs to determine at what point the values had reached stationarity. The parameter values and bipartition posteriors were similar for the four independent runs; therefore all 175,515 post-burnin trees were used. The proportion of the trees that contained each of the observed bipartitions was used as an estimate of the posterior probabilities (Larget and Simon 1999).

Hypothesis Testing. Three *a priori* hypotheses (H_0) were tested against the tree estimates obtained from the observed sequence data set: (1) Dendrobatidae is part of Ranoidea (Griffiths 1959; Duellman and Trueb 1986; Ford and Cannatella 1993; Ford 1993), (2) monophyly of Hylidae + Pseudidae + Centrolenidae (Lynch 1973; Duellman and Trueb 1986; Ford and Cannatella 1993), and (3) *Brachycephalus* is part of Bufonidae (Noble 1931; Griffiths 1959; Lynch 1973). We used the parametric bootstrap test to compare the best tree score from the observed data (H_A) to the best tree score obtained from a topology constrained to represent H_0 (Goldman et al. 2000; Huelsenbeck et al. 1996; Buckley 2002). The observed dataset was used to calculate the difference (H_0–H_A) between the shortest tree score under the null hypothesis and the shortest tree score under the alternative hypothesis. A null distribution of tree length differences was generated by simulating 500 datasets (SeqGen, V. 1.2.5.) using the model of evolution which best described the observed sequence data under the null hypothesis. For each simulated data set, the difference in tree scores under H_0 and H_A was calculated. These 500 differences comprised the expected difference to which the observed difference was then compared. If the observed difference was greater than 95% of the 500 differences computed from

the simulated data sets, then the observed difference was judged to be significantly different from the null distribution, and therefore, the null hypothesis was rejected.

1.3 RESULTS

Parsimony Analysis. Unweighted parsimony analysis of the 2,001 included characters (of which 1,040 were parsimony-informative; 498 ambiguous sites were excluded from the analysis) yielded three most-parsimonious reconstructions each with a score of 11,763 steps, CI = 0.198 and RI = 0.436 (Figure 1.1). All three trees supported a monophyletic Hyloidea (Hylidae, Leptodactylidae, Bufonidae, Centrolenidae, Pseudidae, and Brachycephalidae), and monophyletic Ranoidea ("Ranidae", Microhylidae, Hyperoliidae, Rhacophoridae), with high non-parametric bootstrap values (bp) of 92 and 96 respectively (Figure 1.1). Between Hyloidea and Ranoidea, uncorrected sequence divergence varied from 15% to 27%, and within-Hyloidea sequence divergence reached 23%. Non-parametric bootstrap resampling revealed that no interfamilial relationships within Hyloidea have support values greater than 50%. Three monophyletic hyloid families were recovered: Dendrobatidae, Bufonidae and Centrolenidae (bp = 99, 35, and 100).

Although relationships within Ranoidea are not the focus of these analyses, our limited taxon sampling recovered three major clades: one with ranine ranids, platymantine ranids, and Rhacophoridae; another with brevicipitine microhylids,

Hyperoliidae, and *Hemisus*; and a third composed of the remaining microhylids. This renders Microhylidae nonmonophyletic.

Hylidae is polyphyletic. Pseudidae, as represented by *Pseudis paradoxa,* is most closely related to the hyline *Scarthyla goinorum* (bp = 97). The two representatives of the hylid subfamily Hemiphractinae, *Cryptobatrachus* sp. and *Gastrotheca pseustes*, are the sequential sister-groups to the clade containing all hyloids except *Brachycephalus* and the eleutherodactylines, but this relationship is poorly supported (bp < 50).

Brachycephalidae, as represented by *Brachycephalus ephippium,* is most closely related to a clade of Mexican and Central American members of the leptodactylid tribe Eleutherodactylini, including *Hylactophryne augusti, Eleutherodactylus fitzingeri,* and *E. rhodopis* (bp = 62). The clade containing *Brachycephalus* and all members of Eleutherodactylini appears as the sister group to the rest of Hyloidea (bp = 59). This renders Leptodactylidae polyphyletic; the family is represented on the parsimony tree by five clades.

Maximum Likelihood and Bayesian Inference Analyses. MODELTEST determined that the best-fit model for our data was GTR+Γ+I. Under this model, the following parameter values were estimated from one of the most parsimonious trees: rate matrix AC 2.71, AG 8.41, AT 3.88, CG 0.57, CT 22.15, GT 1.0; nucleotide frequencies A 0.41, C 0.22, G 0.13, T 0.24; proportion of invariant sites 0.275, gamma distribution shape parameter 0.646.

Maximum likelihood analyses recovered exactly the same topology as was estimated using Bayesian methods, with the exception of one basal hyloid polytomy. Bayesian analyses recovered a polytomy at the most basal hyloid node: (*Cryptobatrachus* sp., *Brachycephalus ephippium* + Eleutherodactylini, the remaining Hyloidea) (Figure 1.2). As in the parsimony analyses, both likelihood and Bayesian methods recovered a monophyletic Hyloidea and Ranoidea, both with Bayesian posterior probabilities (pp) of 100% (Figure 1.2). Again, three major clades of ranoids were recovered, although relationships within these differ slightly from the parsimony results.

Support for the monophyly of the hyloid families Centrolenidae and Dendrobatidae is also 100%. Support for a monophyletic Bufonidae is 99%. As under parsimony, Hylidae is found to be polyphyletic under likelihood and Bayesian analyses, due to the unclear relationships of *Cryptobatrachus* and *Gastrotheca.* Bayesian analyses recovered *Cryptobatrachus* in a polytomy with the clade containing Eleutherodactylini + *Brachycephalus* and the rest of Hyloidea. The likelihood tree placed *Cryptobatrachus* as the sister group to Eleutherodactylini + *Brachycephalus*. *Gastrotheca* appears most closely related to the leptodactylid *Alsodes monticola* (pp = 95%). Again, *Pseudis paradoxa* is most closely related to the hyline *Scarthyla goinorum* (pp = 100%).

The relationship of *Brachycephalus ephippium* and Mexican and Central American eleutherodactylines is strongly supported (pp = 100%). Specifically, *Brachycephalus* is supported as the sister taxa of the Mexican eleutherodactylines (pp =

93%). In addition to the Eleutherodacylini, Leptodactylidae is represented by two clades, one of which includes *Gastrotheca*.

Hypothesis Testing. Parametric bootstrap analyses revealed that the three hypotheses—the placement of Dendrobatidae in Ranoidea, monophyly of Hylidae + Pseudidae + Centrolenidae, and *Brachycephalus* as part of Bufonidae—were rejected by the observed sequence data at $P < 0.002$ (Figure 1.3).

1.4 DISCUSSION

Phylogenetic taxonomy. Our phylogenetic definition of Hyloidea provides a stable name for a strongly supported clade. This definition excludes *Heleophryne*, Myobatrachidae, Limnodynastidae, and Sooglossidae from the definition of Hyloidea. A re-analysis of the data from Ruvinsky and Maxson (1996) and Hay et al. (1995), as well as our unpublished results, indicate that the relationships among these basal neobatrachian clades are not stable.

We here associate the name Hyloidea with a less inclusive and more stable clade, specifically the most recent common ancestor of Eleutherodactylini, Bufonidae, Centrolenidae, Phyllomedusinae, Pelodryadinae, and Ceratophryinae. Because all our analyses indicate high confidence in this slightly more restricted clade, and other analyses have also found it to be well supported, (Hay et al.1995; Ruvinsky and Maxson 1996; Vences et al. 2000) we recognize this clade formally. If *Heleophryne*, Sooglossidae,

Myobatrachidae, or Limnodynastidae are later found to be nested within Hyloidea, then the definition of Hyloidea will not change.

Ford and Cannatella (1993) defined Ranoidea as "the common ancestor of hyperoliids, rhacophorids, ranids, dendrobatids, *Hemisus*, arthroleptids, microhylids, and all of its descendants." In retrospect, their inclusion of Dendrobatidae in the definition of Ranoidea was unfortunate because its relationships were historically labile. Based on our analysis, two actions are possible: 1) adherence to the original definition, which would drastically expand the content of Ranoidea to include another 3100 species, because the last ancestor of Ranoidea as originally defined now subtends a much larger clade; 2) re-define the name Ranoidea, using reference taxa that provide a more stable definition. In expectation of a more extensive analysis of ranoids, we choose a third option and defer from re-defining the name Ranoidea.

Alternatives to naming the entire clade as Ranoidea should be considered. Our analysis and that of Emerson et al. (2000) indicate three well-supported clades: (1) one of rhacophorids, Mantellinae, and traditional "ranids" such as *Rana* and *Platymantis*; (2) one of most groups of microhylids; and (3) one of Arthroleptidae, Hyperoliidae, *Hemisus* (in Hemisotidae), and brevicipitine microhylids. The oldest available Linnean superfamily name for the clade of ranids, mantellines, and rhacophorids is Ranoidea. The oldest available Linnean superfamily name for the clade of microhylids excluding Brevicipitinae is Microhyloidea. There seems to be no available superfamily name for the third clade; the oldest available genus name in this clade is *Breviceps* Merrem 1920.

Thus, the superfamily name would be Brevicipitoidea; its author and date would derive from Brevicipitinae Bonaparte 1850.

Hypothesis testing. Our tests yielded new insights into long-standing controversies in anuran systematics. The position of Dendrobatidae has long been debated. Noble (1926, 1931) suggested that dendrobatids were associated with the hylodine leptodactylids based on the presence of digital dermal scutes and the morphology of the pectoral girdle. Lynch (1971, 1973) also strongly supported this hypothesis. Griffiths (1959) proposed placing Dendrobatidae with the ranoids based mostly on features of the pectoral girdle and thigh musculature. The dendrobatid-ranoid hypothesis was further fueled by Duellman and Trueb (1986), Ford and Cannatella (1993), and Ford (1993). Three molecular studies found Dendrobatidae to be associated with hyloid families and excluded from the cluster of ranoid families (Hay et al. 1995; Ruvinsky and Maxson 1996; Vences et al. 2000). With a four-fold increase in non-dendrobatid neobatrachian taxa, our placement of Dendrobatidae is concordant with previous molecular analyses.

Using parametric bootstrap simulation we rejected the placement of Dendrobatidae within Ranoidea, $P < 0.002$. However, the systematic affinities of Dendrobatidae within Hyloidea are still unresolved. Parsimony placed Dendrobatidae closest to the hyline *Scinax*, whereas Bayesian and maximum likelihood placed it as the sister group to a clade of some telmatobiine leptodactylids and *Gastrotheca*. Haas (2003) found dendrobatids to be closely related to hylodine leptodactylids, but we had no molecular sequences of hylodines.

Biogeographically, the placement of dendrobatids with hyloids seems more in accord with the observation that hyloids are primarily Neotropical, whereas under the "dendrobatids as ranoids" hypothesis, Dendrobatidae was the only large radiation of firmisternal frogs in the Neotropics, aside from the lesser invasion of the Neotropics by *Rana* from North America.

Pseudis (Pseudidae) was formerly placed in the Hylidae or Leptodactylidae until it was elevated to family level by Savage and Carvalho (1953) based on the presence of a large intercalary element in each digit. Lynch (1973), Duellman and Trueb (1986), and Ford and Cannatella (1993) used this character to unite the hylids, centrolenids and pseudids. Hay et al. (1995), however, found Pseudidae to be the sister taxon to a clade including Dendrobatidae, Rhinodermatidae, Bufonidae, Hylidae and Centrolenidae. Upon adding eight new neobatrachian taxa to the Hay et al. (1995) data matrix, Ruvinsky and Maxson (1996) found Pseudidae and Rhinodermatidae in a weakly-supported trichotomy with Pelodryadinae+Phyllomedusinae. At $P < 0.002$, we were able to reject the monophyly of the clade containing Hylidae, Pseudidae and Centrolenidae.

Both parsimony and Bayesian analyses recovered *Pseudis paradoxa* as most closely related to the hyline *Scarthyla goinorum* (bp = 97; pp = 100%). Like pseudids, this hylid (originally *S. ostinodactyla*) has ossified intercalary elements between the penultimate and distal phalanges (Duellman and de Sá 1988). As in our analyses, da Silva (1998: Figure II-7) placed *Scarthyla* as the sister-taxon of (*Pseudis* + *Lysapsus*), nested within hylines. However, his morphological data indicate that the presence of

calcified intercalary elements is not a synapomorphy for *Scarthyla* + Pseudidae; rather, this character appears deeper in his tree and is homologous among pseudids, *Scarthyla*, some *Sphaenorhynchus*, and *Pseudacris*.

Based on da Silva (1998), Duellman (2001) argued that pseudid frogs should be recognized as a subfamily of Hylidae, and he figured Pseudinae as the sister taxon to Hylinae (Duellman 2001:Figure 331). However, da Silva (1998) intimated that pseudids should be placed within Hylinae (rather than in Pseudinae), given that Pseudinae was nested within hylines, but he stopped short of a formal taxonomic change. Because our results place *P. paradoxa* within Hylinae, ranking pseudids as either family or subfamily (Pseudidae or Pseudinae) still renders Hylidae or Hylinae paraphyletic, which is inconsistent with the principles of phylogenetic taxonomy (de Queiroz and Gauthier 1992). Therefore, within the Linnean framework, we consider the names Pseudidae and Pseudinae to be junior subjective synonyms of Hylidae.

Brachycephalus and *Psyllophryne* (Brachycephalidae) are endemic to the Atlantic forest of southeastern Brazil and are characterized by their tiny size and reduced number of phalanges in the hands and feet. *Brachycephalus* has generally been considered to be related to hyloids, specifically bufonids (Noble 1926, 1931; Griffiths 1959). McDiarmid (1971) removed *Brachycephalus* from Bufonidae based on the absence of a Bidder's organ and elevated the genus to its own family, Brachycephalidae. Izecksohn (1971, 1988) hypothesized a close relationship of *Euparkerella* to *Brachycephalus* and *Psyllophryne*. *Euparkerella* is a diminutive member of the leptodactylid tribe

Eleutherodactylini, which like *Brachycephalus* and *Psyllophryne,* lives in leaf litter in the forests of southeastern Brazil.

Using parsimony, maximum likelihood, and Bayesian analysis, we recovered a close association between *Brachycephalus ephippium* and Eleutherodactylini, especially those species in Mexico and Central America. It is surprising that *Brachycephalus* is allied to Central American and Mexican species rather than to South American species; however, our sample of eleutherodactylines is limited.

We were able to reject the null hypothesis that *Brachycephalus* is a bufonid using parametric bootstrap analysis ($P < 0.002$). Our results strongly support Izecksohn's (1988) hypothesis that *Brachycephalus* is most closely related to Eleutherodactylini. Inclusion of *Brachycephalus* in Eleutherodactylini would nest a family (Brachycephalidae) within a tribe, which is inconsistent with Linnean taxonomy. This arrangement also forces Eleutherodactylini to be paraphyletic and is inconsistent with the principles of phylogenetic taxonomy (de Queiroz and Gauthier 1992). Therefore, continued recognition of a family-group name based on the type-genus *Brachycephalus* is unwarranted. However, the nomenclatural implications of synonymization of Brachycephalidae are extensive and will be treated elsewhere (Cannatella and Darst in prep).

Other relationships. All phylogenetic methods recovered a monophyletic Hyloidea and Ranoidea. We found, however, topological and nodal support incongruences between parsimony and model-based methods for basal hyloid relationships. The weak bootstrap

support for the deep hyloid divergences most probably comes from a combination of apparent short divergence times on internal branches (Figure 1.2) with possible substitutional saturation.

Bayesian analyses estimated much higher support values than did parsimony. Bootstrap proportions are known to be highly conservative (Hillis and Bull 1993), whereas the higher levels of support seen in posterior probabilities reflect a closer measure of phylogenetic accuracy (Wilcox et al. 2002; but see Suzuki et al. 2002). However, the support values from non-parametric bootstrapping and Bayesian analyses are not strictly comparable because bootstrap values were calculated under parsimony whereas the Bayesian analyses used a likelihood function.

1.5 CONCLUSIONS

Our analysis of 12S, tRNA-valine, and 16S rRNA mitochondrial genes from 93 neobatrachian taxa provides statistically significant support for a monophyletic Hyloidea and Ranoidea. Some new patterns of hyloid phylogenetic relationships were uncovered. First, monophyly of Centrolenidae, Bufonidae, and Dendrobatidae, is strongly supported by parsimony, maximum likelihood and Bayesian analyses. Also, we explicitly rejected the hypothesis that the Dendrobatidae is most closely related to ranoid taxa. Second, Hylidae is polyphyletic. Specifically, *Cryptobatrachus* sp. and *Gastrotheca pseustes* (Hemiphractinae) do not appear closely related to each other, nor to other hylids; greater

taxon sampling is needed. Third, a clade of Hylidae, Pseudidae and Centrolenidae was not recovered and we explicitly rejected the monophyly of this clade using parametric bootstrapping. Using both parsimony and Bayesian analysis, Centrolenidae appears to be most closely related to leptodactyline leptodactylids. *Pseudis paradoxa* and the hylid *Scarthyla goinorum* form a well-supported clade. This position of *P. paradoxa* within Hylinae supports synonymization of Pseudidae (and Pseudinae). Lastly, we rejected the hypothesis that *Brachycephalus* is most closely related to Bufonidae. Rather, it is most closely related to the leptodactylid tribe Eleutherodactylini, especially species from Central America and Mexico.

Table 1.1. Primers used to amplify and sequence 12S, tRNA-val and 16S rRNA mitochondrial genes.

Primer name	Primer sequence 5' to 3'	Position[a]	Goebel No.[b]
MVZ59	ATAGCACTGAAAAYGCTDAGATG →	2153–2180	29
tRNAphe	GCRCTGAARATGCTGAGATGARCCC →	2161–2185	30
12L1	AAAAAGCTTCAAACTGGGATTAGATACCCCACTAT →	2475–2509	46
12SM	GGCAAGTCGTAACATGGTAAG →	2968–2989	--
tRNAval	GGTGTAAGCGAGAGGCTT ←	3033–3059	73
MVZ50	TCTCGGTGTAAGCGAGAAACTT ←	3042–3063	72
16SH	GCTAGACCATKATGCAAAAGGTA ←	3282–3304	76
16SC	GTRGGCCTAAAAGCAGCCAC →	3623–3642	--
16SA	ATGTTTTTGGTAAACAGGCG ←	3956–3976	87
16SD	CTCCGGTCTGAACTCAGATCACGTAG ←	4549–4574	--

[a] Roe, B.A., Ma, D.P., Wilson, R.K., & Wong, J.F. 1985. The complete nucleotide sequence of the *Xenopus laevis* mitochondrial genome. J. Biol. Chem. 260, 9759–9774.

[b] Primers with no designated number were designed in the Cannatella lab, not modified from Goebel et al. 1999.

Table 1.2. List of specimens examined. ICN: Instituto de Ciencias Naturales, Universidad Nacional de Colombia; KU: University of Kansas; MVZ: Museum of Vertebrate Zoology; PNM/CMNH: Philippines National Museum/Cincinnati Museum of Natural History; QCAZ: Quito-Católica-Zoologia; TNHC: Texas Natural History Collection; USNM: United States National Museum; USP: Universidade de São Paulo; UTACV: University of Texas at Arlington Collection of Vertebrates.

Family	Species	Field number	Museum number	GenBank number	Locality
Brachycephalidae	*Brachycephalus ephippium*	DMH #2	Not Available (NA)	AY326008	Brazil
Bufonidae	*Ansonia* sp.	H1473	PNM/CMNH	AY325992	Philippines: Mindanao: S. Cotobat Province, Municipality of Kiamba, Mt. Busa
	Atelopus varius	AG 36	MVZ 223279	AY325996	Costa Rica: South of Las Alturas
	Bufo alvarius	DCC 2906	TNHC 61247	AY325984	Arizona: Just north of Tucson
	Bufo biporcatus	DCC 2914	TNHC 61079	AY325987	No data
	Bufo boreas	RDS 239	NA	AY325983	No data
	Bufo bufo	DMH 89-13	TNHC 56744	AY325988	USSR: Latvian Republic, Riga
	Bufo exsul	FC12574	MVZ 142947	AY325990	California: Inyo: 0.8 mi S. Deep Springs College, Bucklehorn Spring, Deep Springs Valley
	Bufo kisoloensis	AG 46	MVZ 223361	AY325995	Uganda: Buhoma, Bwindi Forest Reserve
	Bufo marinus	WED 55596	KU 205236	AY325994	Peru: Madre de Dios: Cusco Amazónico
	Bufo microscaphus	RDJ 865	NA	AY325989	New Mexico: Catron: Bull Pass Tank, 5 mi N,

Family	Species	Field number	Museum number	GenBank number	Locality
					35.5 mi W of Winston; T10S, R14W, Sec 27
	Bufo retiformis	AG 125	MVZ 222506	AY325982	Arizona: Pima: 12 mi N of Quijotoa, Indian Route 15
	Bufo steindachneri	AG 61	MVZ 223373	AY325981	Kenya: Arobuko Sokoka forest, sand quarry
	Bufo nebulifer	DCC 3107	TNHC 62000	AY325985	Texas: San Saba: Colorado Bend State Park
	Dendrophryniscus minutus	USNM-FS 189767	USNM 520905	AY326000	Peru: Loreto: Rio Lagarto Cocha, Aguas Negras
	Didynamipus sjostedti	AG 259	NA	AY325991	Cameroon
	Melanophryniscus sp.	RMB 4125	TNHC 62494	AY325998	No data
	Melanophryniscus stelzneri	AG 87	NA	AY325999	No data
	Osornophryne guacamayo	AGG 220	QCAZ 4580	AY326036	Ecuador: Napo: Lago Sumaco, Volcán Sumaco
	Pedostibes hosei	JAM 1159	NA	AY325993	Malaysia: Pahang: Krau Wildlife Reserve, Pehang main research field station, ~13 km NW Kuala Krau at confluence Krau and Lompat Rivers
	Schismaderma carens	DCC 3172	TNHC 62001	AY325997	Tanzania: Dodoman
Centrolenidae	*Cochranella* sp.	WED 53034	KU 202801	AY326025	Ecuador: Carchi: ~5 km W La Gruel, 2340 m
	Centrolene sp.	WED 52978	KU 202796	AY326022	Ecuador: Napo: 18 km E Santa Bárbara
	Cochranella sp.	AGG 507	QCAZ 10801	AY326023	No data

Family	Species	Field number	Museum number	GenBank number	Locality
	Hyalinobatrachium sp.	RMB 4126	TNHC 62495	AY326024	No data
Dendrobatidae	*Allobates femoralis*	WED 55470	KU 205291	AY326026	Peru: Madre de Dios: Cusco Amazónico
	Allobates femoralis	WED 55560	KU 205292	AY326027	Peru: Madre de Dios: Cusco Amazónico
	Colostethus infraguttatus	AGG 504	QCAZ 10812	AY326028	Ecuador: Manabi: 12 km al norte de Puerto Cayo
	Dendrobates auratus	DCC 2895	TNHC 62487	AY326036	No data
	Dendrobates reticulatus	DCC 3155	TNHC 61143	AY326029	Peru
	Phyllobates bicolor	DCC 2907	TNHC 62488	AY326031	No data
Heleophrynidae	*Heleophryne purcelli*	DMH #15	NA	AY326072	South Africa
Hemisotidae	*Hemisus marmoratum*	DCC 3047	TNHC 62489	AY326070	Tanzania: Arusha near Mt. Kilamanjaro
Hylidae	*Agalychnis litodryas*	CP13217	QCAZ 13217	AY326043	Ecuador
	Agalychnis saltator	DCC 2132	MVZ 203768	AY326044	Costa Rica: Heredia: Starkey's Woods, 1.5-3.0 km E Rio Frio rd at 1 km NW entrance to Estación Biológica La Selva
	Cryptobatrachus sp.	JDL 14865	ICN	AY326050	Colombia: Santander: Municipio San Gil: 7 km by road SW San Gil
	Gastrotheca pseustes	DMH 90E-19	TNHC 62492	AY326051	Ecuador: Chimborazo: 3.3 km S Tixán, 2990 m
	Hyla calcarata	WED 54086	KU 202911	AY326056	Ecuador: Napo: Misahualli, 600 m

Family	Species	Field number	Museum number	GenBank number	Locality
	Hyla lanciformis	WED 54081	KU 202724	AY326054	Ecuador: Pastaza: 5.6 km N Puyo, 1150 m
	Hyla pantosticta	WED 52976	KU 202732	AY326052	Ecuador: Napo: 18 km E Santa Barbara
	Hyla picturata	WED 53656	KU 202737	AY326055	Ecuador: Pichincha: Tinalandia, 15.5 km SE Santo Domingo de Colorados, 700 m
	Hyla sp.	WED 53493	KU 202760	AY326057	Ecuador: Azuay 2.0 km SSE Palmas, 2340 m
	Hyla triangulum	WED 54094	KU 202745	AY326053	Ecuador: Napo: Misahualli, 600 m
	Hyla pellucens	WED 53621	KU 202734	AY326058	Ecuador: Pichincha: 1.8 km SSE San Juan, 3420 m
	Litoria arfakiana	CCA 503	TNHC 51936	AY326039	Papua New Guinea: Madang: ~10 km NW Simbai, Kaironk Village, 2000 m
	Nyctimystes kubori	CCA 496	TNHC 51924	AY326037	Papua New Guinea: Madang: ~10 km NW Simbai, Kaironk Village, 2000 m
	Osteocephalus taurinus	WED 55452	KU 205406	AY326041	Peru: Madre de Dios: Cusco Amazónico
	Pachymedusa dacnicolor	FC12110	MVZ 164906	AY326047	Mexico: Michoacán: Capirio, Rio Tepalcatepec
	Pelodryas caerulea	DMH	NA	AY326038	No data
	Phrynohyas venulosa	DCC 3069	TNHC 62490	AY326048	Ecuador
	Phyllomedusa palliata	WED 55638	KU 205420	AY326046	Peru: Madre de Dios: Cusco Amazónico
	Phyllomedusa tomopterna	WED 55380	KU 205428	AY326045	Peru: Madre de Dios: Cusco Amazónico

Family	Species	Field number	Museum number	GenBank number	Locality
	Pseudacris brachyphona	ECM 41	TNHC 62304	AY326049	Alabama: Tallapoosa Co.
	Scarthyla goinorum	WED 55411	KU 205763	AY326035	Peru: Madre de Dios: Cusco Amazonico
	Scinax garbei	WED 54071	KU 202764	AY326033	Ecuador: Chimborazo: 6.7 km E Riobamba, 2550 m
	Scinax rubra	WED 56265	KU 207622	AY326034	Peru: Madre de Dios: Cusco Amazónico
	Smilisca phaeota	DMH 86-115	NA	AY326040	Costa Rica: Limón: Estación Experimental La Lola
	Trachycephalus jordani	DCC 2917	TNHC 61092	AY326042	Ecuador
Hyperoliidae	*Hyperolius* sp.	DCC 3159	TNHC 61197	AY326069	Tanzania
Leptodactylidae	*Alsodes monticola*	NB #2	NA	AY326016	Chile
	Ceratophrys cornuta	WED 55587	KU 202561	AY326014	Peru: Madre de Dios: Cusco Amazónico
	Ceratophrys ornata	DMH A6	NA	AY326013	No data
	Eleutherodactylus chloronotus	WED 52959	KU 202325	AY326007	Ecuador: Napo: 3.5 km E Santa Barbara
	Eleutherodactylus cuneatus	SBH 172809	NA	Y10944	Cuba: Cienfuegos Province, Soledad
	Eleutherodactylus duellmani	WED 53050	KU 202404	AY326003	Ecuador: Carchi: ~5 km W La Gruel, 2340 m
	Eleutherodactylus fitzingeri	DMH 86-112	NA	AY326001	Costa Rica: Limon: Estación Experimental La Lola
	Eleutherodactylus rhodopis	JAC 8492	UTACV A-12957	AY326006	Mexico: Hidalgo: 4.5 km NE Tlanchinol

Family	Species	Field number	Museum number	GenBank number	Locality
	Eleutherodactylus sp.	WED 52979	KU 202623	AY326002	Ecuador: Napo: 18 km E Santa Barbara
	Eleutherodactylus supernatis	WED 52961	KU 202432	AY326005	Ecuador: Napo: 3.5 km E Santa Barbara
	Eleutherodactylus thymelensis	WED 53004	KU 202519	AY326009	Ecuador: Carchi: 12 km W Tufino, 3520 m
	Eleutherodactylus w-nigrum	WED 53045	KU 205076	AY326004	Ecuador: Carchi: ~5 km W La Gruel, 2340 m
	Hylactophryne augusti	JAC 8191	UTACV A-12980	AY326011	Mexico: Jalisco: 2.4 km NW Tapalpa
	Lepidobatrachus sp.	DCC 2915	TNHC 62497	AY326019	No data
	Leptodactylus pentadactylus	FC13095	MVZ 233238	AY326017	Costa Rica: Limón: Rio Pentencia, 2 mi N Tortuguero
	Lithodytes lineatus	N. Basso	USP 968438	AY326012	Brazil: Apiacás
	Phrynopus sp.	WED 52998	KU 202652	AY326010	Ecuador: Carchi: 13.6 km W El Carmelo, 3080m
	Physalaemus nattereri	AJC 95-267	NA	AY326020	Brazil: São Paulo: Luiz Antonio
	Physalaemus riograndensis	AJC 95-233	NA	AY326021	Brazil: Rio Grande do Sul: El Dorado
	Telmatobius niger	DMH 90E-36	TNHC 62493	AY326015	Ecuador: Azuay: 48.8 km WNW Cuenca, 3380 m.
	Telmatobius vellardi	WED 53381	KU 202679	AY326018	Ecuador: Azuay: 10 km NE Girón, 2750 m
Microhylidae	*Callulina kreffti*	DCC 3162	TNHC 62491	AY326068	Tanzania: Mazumbai
	Gastrophryne olivacea	DCC 3106	TNHC 61952	AY326066	Texas: San Saba: Colorado Bend State Park

Family	Species	Field number	Museum number	GenBank number	Locality
	Kaloula conjuncta	RMB 2252	PNM/CMNH	AY326064	Philippines: Negros Island: city of Dumaguete
	Nelsonophryne aequatorialis	WED 53386	KU 202919	AY326067	Ecuador: Loja: 3.7 km S Saraguro, 2800 m
	Phrynomerus sp.	DCC 2901	TNHC 61077	AY326065	No data
Myobatrachidae	*Limnodynastes salminii*	DCC 2898	TNHC 61075	AY326071	No data
Pseudidae	*Pseudis paradoxa*	DCC 3284	NA	AY326032	Brazil: São Paulo: Fazenda Santa Helena, ~18 km S Luiz Antonio
Ranidae	*Platymantis* sp.	JF 0131	NA	AY326061	Solomon Islands
	Rana nicobariensis	RMB 2086	TNHC 59856	AY326062	Indonesia: Jawa Barat: Java Is.: Desa Cikopo; 6º40'19"S, 106º52'42"E
	Rana temporaria	DMH	NA	AY326063	No data
Rhacophoridae	*Philautus acutirostris*	RMB 589	TNHC 59857	AY326059	Philippines: Davao City Prov.: Mindanao Is.: Eagle Foundation Inc. (PEFI) Malagos Eagle camp
	Rhacophorus monticola	RMB 1236	NA	AY326060	Indonesia: Sulawesi Is.: S. Sulawesi: Mt. Lompo Batang: 1580 m

Figure 1.1. Maximum parsimony phylogram rooted with *Limnodynastes salminii* (Myobatrachidae) and *Heleophryne purcelli* (Heleophrynidae). Numbers above branches indicate nonparametric bootstrap values based on 1000 pseudoreplicates. Hyloid clades are labeled with family, subfamily, or tribe name. Families included are

Brachycephalidae, Leptodactylidae (includes subfamilies: Telmatobiinae [including the tribe Elutherodactylini], Leptodactylinae, and Ceratophryinae), Centrolenidae, Bufonidae, Pseudidae, and Hylidae (includes subfamilies Hemiphractinae, Hylinae, Pelodryadinae and Phyllomedusinae).

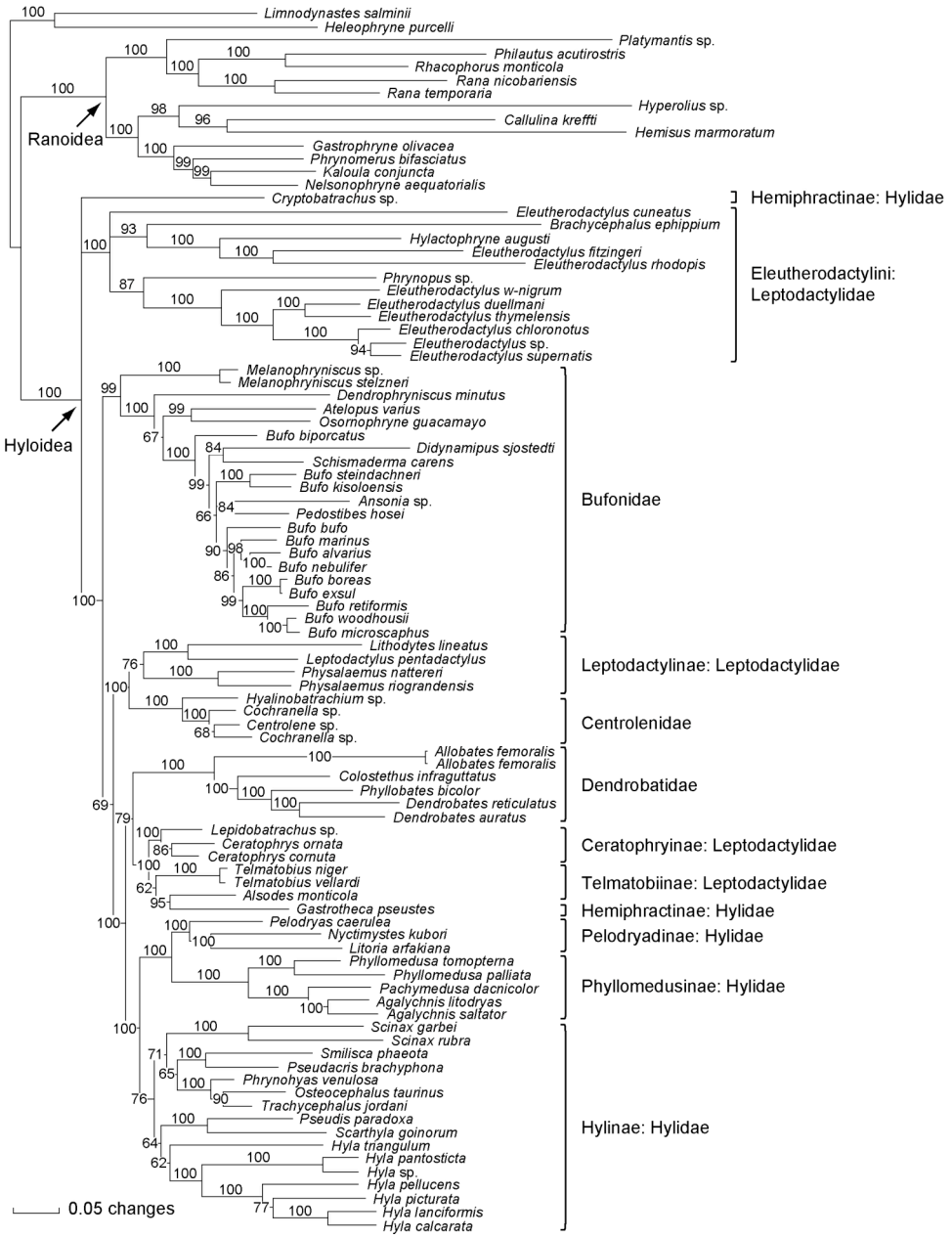

Figure 1.2. Maximum likelihood phylogram under a GTR+Γ+I model of evolution. Numbers above branches indicate posterior probabilities recovered from the Bayesian analysis. Hyloid clades are labeled as in Figure 1.1.

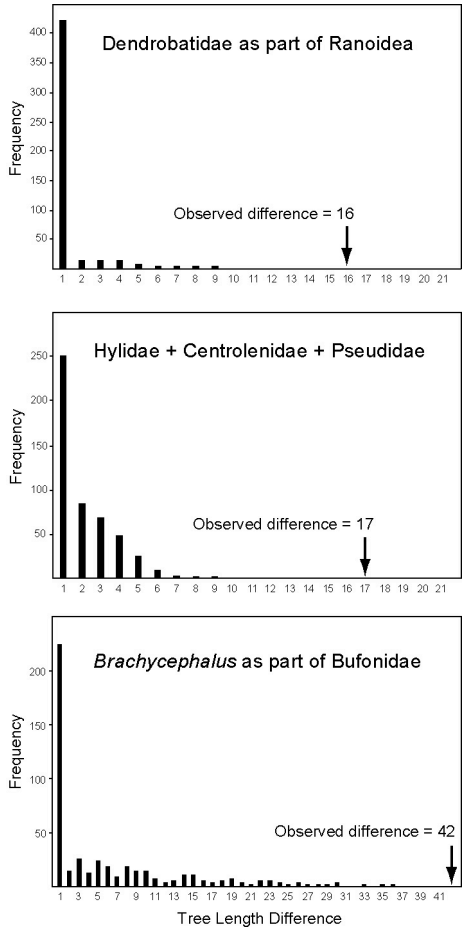

Figure 1.3. Null distributions for the parametric bootstrap test. All observed tree length differences fall outside of their respective null distribution and are therefore significant at $P < 0.002$.

References

Buckley, T. R. 2002. Model misspecification and probabilistic tests of topology: evidence from empirical data sets. Systematic Biology 51: 509–523.

Cannatella, D. C. 1985. A phylogeny of primitive frogs (archaeobatrachians). Ph.D. Dissertation, The University of Kansas, Lawrence.

Da Silva, H. R. 1998. Phylogenetic relationships of the family Hylidae with emphasis on the relationships within the subfamily Hylinae (Amphibia: Anura). Ph.D. Dissertation, The University of Kansas, Lawrence.

De Queiroz, K. & Gauthier, J. 1992. Phylogenetic taxonomy. Annual Review of Ecology and Systematics 23: 449–480.

Dubois, A. 1983. Classification et nomenclature supragénérique des amphibians anoures. Bulletin de la Societe Linneene de Lyon 52: 270–276.

Duellman, W. E. 1975. On the classification of frogs. Occasional Papers of the Museum of Natural History, University of Kansas 42: 1–14.

Duellman, W. E. 2001. *Hylid Frogs of Middle America, Volume 2.* New York: Society for the Study of Reptiles and Amphibians.

Duellman, W. E. & de Sá, R. O. 1988. A new genus and species of South American hylid frog with highly modified tadpole. Tropical Zoology 1: 117–136.

Duellman, W.E. & Trueb, L. 1986. *Biology of Amphibians*. New York: McGraw-Hill Book Co.

Emerson, S. B., Richards, C., Drewes, R. C., & Kjer, K. M. 2000. On the relationships among Ranoid frogs: a review of the evidence. Herpetologica 56: 209–230.

Felsenstein, J. 1985. Confidence limits on phylogenies: a justification. Evolution 39: 783–791.

Ford, L. S. 1989. The phylogenetic position of poison-dart frogs (Dendrobatidae): reassessment of the neobatrachian phylogeny with commentary on complex character systems. Ph.D. Dissertation. The University of Kansas, Lawrence.

Ford, L. S. 1993. The phylogenetic position of the dart-poison frogs (Dendrobatidae) among anurans: an examination of the competing hypotheses and their characters. Etholgoy Ecology and Evolution 5: 219–231.

Ford, L. S. & Cannatella D. C. 1993. The major clades of frogs. Herpetological Monographs 7: 94–117.

Frost, D. R. 1985. *Amphibian Species of the World*. Allen Press, Lawrence.

Frost, D. R. 2002. Amphibian Species of the World: An Online Reference.V2.21 (15 July 2002). Electronic database available at research.amnh.org/herpetology/amphibia/index.html.

Goebel, A. M, Donnelly J. M., & Atz, M. E. 1999. PCR primers and amplification methods for 12S ribosomal DNA, the control region, cytochrome oxidase I, and cytochrome b in bufonoids and all other frogs, an overview of PCR primers which have amplified DNA in amphibians successfully. Molecular Phylogenetics and Evolution 11: 163–199.

Goldman, N., Anderson, J. P., & Rodrigo, A. G. 2000. Likelihood base tests of topologies in phylogenetics. Systematic Biology 49: 652–670.

Griffiths, I. 1959. The phylogeny of *Sminthillus limbatus* and the status of Brachycephalidae (Amphibia, Salientia). Proceedings of the Zoological Society of London 132: 457–487.

Haas, A. 2003. Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura). Cladistics 19: 23-89.

Hay, J. M., Ruvinsky, I., Hedges, S. B., & Maxson, L. R. 1995. Phylogenetic relationships of amphibian families inferred from DNA sequences of mitochondrial 12S and 16S ribosomal RNA genes. Molecular Biology and Evolution 12: 928–937.

Hillis, D. M. & Bull, J. J. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology 42: 182–192.

Huelsenbeck, J. P., Hillis, D. M., & Nielsen, R. 1996. A likelihood-ratio test of monophyly. Systematic Biology 45: 546–558.

Huelsenbeck, J. P. & Ronquist, F. R. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17: 754–755.

Izecksohn, E. 1971. Novo gênero e nova especie de Brachycephalidae do Estado do Rio de Janeiro, Brasil (Amphibia-Anura). Revista Brasileira de Biologia 48: 59–74.

Izecksohn, E. 1988. Algumas considerações sobre o gênero *Euparkerella*, com a descrição de tres novas especies (Amphibia, Anura, Leptodactylidae). Revista Brasileira de Biologia 48: 59–74.

Kluge, A. G. & Farris, J. S. 1969. Quantitative phyletics and the evolution of anurans. Systematic Zoology 18: 1–32.

Larget, B. & Simon, D. 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Molecular Biology and Evolution 16: 750–759.

Laurent, R. F. 1986. Sous classe des lissamphibiens. Systématique, in P.-P. Grassé, and Delsol, M. (Eds.), *Traité de Zoologie*, Masson, Paris, pp. 594–797.

Lynch, J. D. 1971. Evolutionary relationships, osteology, and zoogeography of leptodactylid frogs. Miscellaneous Publications of the Museum of Natural History University of Kansas 53: 1–238.

Lynch, J. D. 1973. The transition from archaic to advanced frogs, in Vial, J.L. (Ed), *Evolutionary Biology of the Anurans: Contemporary Research on Major Problems*, University of Missouri Press, pp. 133–182.

Maddison, W. P. & Maddison, D. R. 2000. MacClade: Analysis of Phylogeny and Character Evolution. Version 4.0. Sinauer, Sunderland, Massachusetts.

McDiarmid, R. W. 1971. Comparative morphology and evolution of frogs on the Neotropical genera *Atelopus*, *Dendrophryniscus*, *Melanophryniscus* and *Oreophrynella*. Scientific Bulletin of the Natural History Museum Los Angeles Co. 12: 1–66.

Noble, G. K. 1926. The pectoral girdle of the brachycephalid frogs. American Museum Novitates 230: 1–14.

Noble, G .K. 1931. *The Biology of the Amphibia*. New York: McGraw-Hill Book Company.

Palumbi, S. R. 1996. *Nucleic acids II: The polymerase chain reaction*, in Hillis, D.M., Mable, B.K., and Moritz, C. (Eds.) Molecular Systematics, 2nd ed., Sinauer, Sunderland, Massachusetts, pp. 205–247.

Posada, D. & Crandall, K. A. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817–818.

Reig, O. A. 1958. Proposiciones para una nueva macrosistemática de los anuros. Nota preliminar. Physis, Buenos Aires 21: 109–118.

Ruvinsky, I. &Maxson, L.R. 1996. Phylogenetic relationships among bufonoid frogs (Anura: Neobatrachia) inferred from mitochondrial DNA sequences. Molecular Phylogenetics and Evolution 5: 533–547.

Savage, J. M. & de Carvalho, A. L. 1953. The family position of the Neotropical frogs currently referred to the genus *Pseudis*. Zoologica 38: 193–200.

Suzuki, Y., Glazko, G. V., & Nei, M. 2002. Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. Proceedings of the National Academy of Sciences USA 99: 16138–16143.

Swofford, D. L. 2000. PAUP* Phylogenetic Analysis Using Parsimony (*And Other Methods*)*, Version 4.0b.8a. Sinauer, Sunderland, Massachusetts.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. 1997. The Clustal_X windows interface: flexible strategies for multiple sequence alignment by quality analysis tools. Nucleic Acids Research 25: 4876–4882.

Tihen, J. A. 1965. Evolutionary trends in frogs. American Zoologist 5: 309–318.

Vences, M., Kosuch, J., Lötters, S., Widmer, A., Jungfer, K-H., Köhler, J., & Veith, M. 2000. Phylogeny and classification of poison frogs (Amphibia: Dendrobatidae), based on mitochondrial 16S and 12S ribosomal RNA gene sequences. Molecular Phylogenetics and Evolution 15: 34–40.

Wilcox, T. P., Zwickl, D. J., Heath, T. A., & Hillis, D. M. 2002. Phylogenetic relationships of the dwarf boas and a comparison on Bayesian and bootstrap measures of phylogenetic support. Molecular Phylogenetics and Evolution 25: 361–371.

Chapter 2

Evolution of Toxicity and Dietary Specialization in Poison Frogs (Dendrobatidae): A Comparative Analysis

Abstract: Defense mechanisms, such as toxicity, are favored by predation-driven natural selection. The acquisition of toxicity can be either endogenous, in which the toxins are produced by the organism itself, or exogenous, in the form of sequestered compounds produced by other organisms. It has been suggested that the defensive skin toxins of Neotropical poison frogs (Dendrobatidae) have an exogenous source: a diet of ants and other small arthropods rich in toxic alkaloid chemicals. A critical prediction from the diet-toxicity hypothesis in poison frogs is that independent origins of diet specialization will be found to be correlated with the independent origins of toxicity. We tested this prediction in an integrated framework using comparative methods with new and published data on dietary specialization and toxicity for fifteen species of dendrobatids in five genera. We found a significant correlation between level of toxins and degree of dietary specialization. This reveals a recurring association of toxicity and dietary specialization in dendrobatids, which suggests parallel evolutionary trends in the origins of defense mechanisms.

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2.1 INTRODUCTION

Predation imposes important selective pressures on prey, resulting in a great diversity of defense mechanisms (Edmunds 1974). One of the most intriguing defensive mechanisms is repellent defense, such as venoms and toxins. Toxicity can come from either endogenous synthesis (the organism's metabolic machinery produces the toxic compounds) or exogenous sources (via uptake, sequestration and/or storage of toxic compounds produced by other organisms) (Eisner 1970; Edmunds 1974; Mebs 2001). Toxic or otherwise unpalatable individuals are often brightly colored; this association is called aposematism (Poulton 1890). Presumably, warning coloration would be selected for after the acquisition of toxicity, because any easily learned color or character that identifies the toxic individual to experienced predators would be favored (Cott 1940; Edmunds 1974, 1987).

The acquisition of defensive compounds from the environment raises the possibility of integrating ecology and the evolutionary origin of chemical defense. Although dietary specialization is often regarded as a plastic feature, the role of diet in influencing historical factors of diversification could be important in organisms that acquire their defense from diet. One approach to this question is to search for convergent, and therefore probably adaptive, patterns of resource use across taxa (Strong 1979; Futuyma 1983; Orians and Paine 1983). The role of diet in macroevolutionary processes has been most thoroughly studied in insects (Ehrlich and Raven 1964; Berenbaum 1983; Jeffries and Lawton 1984; Feeny 1987; Denno et al. 1990; Farrell and

Mitter 1990; Becerra 1997; Becerra and Venable 1999; Dobler 2001), but has not been extensively addressed in other taxa. The relationship between diet ecology and the sequestration of toxic compounds in the context of the evolutionary history of Neotropical poison frogs (Dendrobatidae) is the focus of this study.

The family Dendrobatidae is composed of 221 small, diurnal species in at least nine genera (updated from Frost 2002). Species of *Colostethus* (plus *Mannophryne* and *Nephelobates* which were extracted from *Colostethus* by La Marca 1992, 1994) are generally non-toxic, cryptically colored dendrobatids, whereas species of *Epipedobates, Phyllobates,* and *Dendrobates* are those with the most toxic skin and brilliant, aposematic coloration (Myers 1987; Myers et al. 1978; Daly et al. 1980, 1987, 1994). The skin of toxic dendrobatids houses a diverse range of lipophilic alkaloids (Daly et al. 1999 and references therein). These alkaloids are noxious to predators and therefore serve a defensive role, although the evidence for this is somewhat anecdotal (Daly and Myers 1967; Lülling 1971; Fritz et al. 1981; Szelistowski 1985). Even though the precise sister group of Dendrobatidae is not clear (Darst and Cannatella, in prep), none of the apparent close relatives derives toxicity from alkaloids. Therefore, we assume that lack of toxicity is primitive for dendrobatids (as also suggested by Caldwell 1996).

The first extensive molecular phylogenetic analysis of 27 dendrobatid species reconstructed a single origin of aposematism (Clough and Summers 2000). *Colostethus* was the most basal taxon, exhibiting the cryptic, non-toxic ancestral state, followed by the transitional, more toxic, brightly colored *Epipedobates*, then culminating in the most

brightly colored, toxic taxa, *Phyllobates* and *Dendrobates*. However, with more extensive taxon sampling, especially in *Colostethus*, several independent origins of aposematism were recovered (Santos et al. 2003; Vences et al. 2003). *Colostethus* was found to be paraphyletic with respect to the more brightly colored *Allobates*, *Cryptophyllobates*, the clade *Phyllobates+Dendrobates* and *Epipedobates*, the last being a polyphyletic assemblage. The multiple origins of aposematism occur at different time scales; aposematism had an ancient origin in *Phyllobates+Dendrobates* (a large group of toxic species), whereas other origins occurred between brightly colored species and cryptic sister taxa that show little genetic divergence.

Evidence is mounting that alkaloids in the skin of poison frogs are accumulated from dietary sources: small, leaf-litter arthropods (Daly et al. 1999, 2000, 2002, 2003; Spande et al. 1999; Saporito et al. 2003). It has been shown that the few species of aposematic dendrobatids examined have specialized diets with a larger percentage of ants, larger number of prey per individual, and smaller niche breadths (Toft 1980, 1995; Donnelly 1991; Simon and Toft 1991; Caldwell 1996; Parmelee 1999); however, these conclusions were made in the context of a single origin of aposematism. Thus, dietary specialization and the evolution of an uptake system for alkaloids was postulated to be a key innovation leading to the development of toxic skin and permitting the evolution of aposematism and diversification in dendrobatids (Caldwell 1996).

Given the recent discovery of multiple origins of aposematism, a critical prediction of this "diet-toxicity" hypothesis is that independent origins of dietary

specialization will be correlated with the independent origins of toxicity. Santos et al. (2003: their Figure 2) inferred at least two and possibly three origins of ant-specialization, but this inference was not based on an explicit analysis. To test the prediction of the diet-toxicity hypothesis in an integrated framework, we used the molecular phylogeny of Santos et al. (2003) and a complex of ecologically relevant traits compiled from 17 species (nine new species and published data for eight species) in a total of five genera. We explore the evolutionary and behavioral ecology of toxin sequestration through quantification of diet contents and dietary niche, and through an indirect assessment of predator defense using an assay for skin alkaloids. To our knowledge, this is one of the first hypothesis-driven tests of an association between diet and defense related to warning coloration in a vertebrate system.

2.2 MATERIALS AND METHODS

Specimen Collection. Fieldwork was conducted in tropical Amazonian lowland rain forest, Western Andean slopes, and Pacific lowlands of Ecuador. The four collection sites were: 1) Estación Científica Yasuní, Orellana Province (*Allobates femoralis*, *Colostethus bocagei*, *C. sauli*, *C. insperatus*, *Epipedobates bilinguis*, and *E. hahneli*), 2) Estación Biológica Jatun Sacha, Napo Province (*A. zaparo*, *E. bilinguis*, *E. hahneli*, and *E. parvulus*), 3) 6 km (airline) WNW Pedro Vicente Maldonado and 9 Km W of Santo Domingo de los Colorados (on road to Chone), Pichincha Province (*E. boulengeri*), and 4) 13.4 km east of Echeandía at 1131 m, Bolívar Province (*E. tricolor*).

Frogs were collected by hand (when possible, in a plastic cup so as not to contaminate the skin by handling) and euthanized by pithing to avoid contamination by chemical agents. The skin and digestive tract were removed and preserved as soon as possible after collection, usually within 1–2 hours, in order to prevent further digestion of consumed prey and to complement Caldwell (1996). Skins were fixed in 100% methanol; digestive organs were stored in 90% ethanol. Specimens were preserved in 10% formalin and transferred to 70% ethanol. Frog voucher specimens and stomach contents are deposited at Museo de Zoología, Pontificia Universidad Católica del Ecuador (QCAZ).

Diet Analysis. Gastrointestinal tract contents were sorted using a dissecting microscope, and prey items were identified to the lowest taxonomic category possible, usually order, although some items (i.e., Hymenoptera, Homoptera, and Coleoptera) were identified to family. The length and width of each intact prey item was measured to 0.01 mm (with digital calipers) and volume was calculated using the formula for a prolate spheroid (Dunhan 1983):

$$V = \frac{4\pi}{3}(length/2)\cdot(width/2)^2$$

Length measurements excluded antennae and ovipositors. Width was recorded at the midpoint of the prey item, excluding appendages. For each species, a histogram of percent of total prey by volume in the 15 most well-represented prey categories was

produced as a general representation of the distribution of prey items in the diet (Figure 2.1).

Diet was quantified using six variables (Table 2.1); three explored general aspects of the diet, and three focused on the importance of ants: the proportion of individuals in each species with ants in the gastrointestinal tract (%INDANTS), the percentage of ants by number in the total prey (%ANTSNUM), the percentage of ants by volume in the total prey (%ANTSVOL), the number of prey per individual frog (NUMPREY), the niche breadth of each species calculated for prey number (NBNUM) and prey volume (NBVOL). Caldwell (1996) computed these same variables for 212 frogs in 8 species, using the protocol described above. Niche breadth was calculated using the inverse of Simpson's (1949) formula:

$$B = 1 \Big/ \sum_{i=1}^{n} p_i^2$$

where i is the resource category, p is the proportion of resource category i, and n is the total number of prey categories (Pianka 1986). Niche breadth values vary from 1.0 (exclusive use of a single prey category) to n (all prey categories used equally).

It has been argued that electivities, which measure the proportion of prey eaten as compared to the prey availability (the proportions of prey in the leaf litter), are more realistic quantifications of dietary specialization than niche breadth (Ivlev 1961; Jacobs

1974; Toft 1995). We did not calculate electivities because Toft (1980) found that categorization of dendrobatids along a specialist–generalist continuum was the same no matter whether niche breadth or electivity values were used.

Skin Chemistry Analyses. As a proxy for direct measures of toxicity to predators, we assessed alkaloid profiles from 130 dendrobatid skins (110 of which belonged to the animals used in diet analyses) using thin layer chromatography (TLC; Myers and Daly 1976). Individual skins were placed into polyethylene NUNC™ vials with 1–1.5 ml 100% methanol. After a few weeks, the skin alkaloids were extracted by the methanol, and skins were removed and stored in fresh methanol and deposited in the collections of QCAZ. A sample of 10 µl of each methanol extract was applied as a small spot to an aluminum-backed silica gel TLC plate ($60F_{265}$; EM Science), followed by development of the plate with a 1:10 mixture of methanol and chloroform. After drying, the plate was placed in a chamber containing iodine crystals and was heated to vaporize the iodine; this allows visualization of alkaloids as orange-brown spots on a light background. Digital photographs of all TLC plates were taken because the iodine vapor produces only a semi-permanent record. Each lane on the TLC plate was scored as positive, negative, or trace for alkaloids. A positive result was recorded when a substantial orange-brown streak, usually containing 2–6 dark spots, appeared upon exposure to the iodine vapor; a negative result was recorded when nothing appeared; and a trace result was recorded only when a very faint, singular spot appeared. Individual extracts of various species were compared side-by-side.

To create total toxicity scores (Total Toxicity; Table 2.2), our TLC data were combined with the data that Summers and Clough (2001) compiled from Daly et al. (1987). We used the total toxicity scores for nine species directly from Summers and Clough with the following exceptions. Because data for *Epipedobates boulengeri* were not available, Summers and Clough (2001) assigned it the same score as its closest relative, *E. espinosai*, which shows moderate toxicity (Daly et al. 1987). In our sample of 20 skin extracts of *E. boulengeri*, no alkaloids were detected, so this species was scored as 0.

Also, Summers and Clough (2001) used toxicity scores for *Epipedobates tricolor* and *E. anthonyi*, as reported by Daly et al. (1987). The populations reported by Daly et al. (1987) differ quite markedly in distribution and abundance of toxins, but they are all referable to *E. anthonyi* based on the localities. The type locality of *E. anthonyi* is southeast of Guayaquil, Ecuador. The type locality of *E. tricolor* is northeast of Guayaquil, and the toxicity of this species has not been assessed. We used the toxicity value of *E. anthonyi* from Pasaje, El Oro, Ecuador as reported by Summers and Clough (2001) as our score for *E. tricolor* because it is less likely to reject the null hypothesis than the other score.

Although we detected no alkaloids in *Allobates femoralis*, we used the published score of 1.2 for that species. Summers and Clough (2001) assigned the next highest score (3.1) to *E. bilinguis*; our TLC data from *Epipedobates bilinguis* were consistent with this score. *Allobates zaparo* displayed at most trace amounts of alkaloids, and side-by-side

comparisons of TLC data for *A. femoralis*, *E. bilinguis*, and *A. zaparo* indicated a score of *A. zaparo* intermediate between the other two; we, therefore, assigned it a Total Toxicity score of 2.0.

We also assigned presence/absence toxicity scores (0 or 1; Table 2.2) to all 15 species (Binary Toxicity). Some researchers (e.g., Daly et al. 2002) have questioned the degree of realism in the total toxicity score of Summers and Clough (2001) and therefore a more conservative measure might be desirable. Using Binary Toxicity, the statistical tests for association between toxicity and diet will be more conservative. Also, most investigators should be able to agree that species can be divided into two groups, toxic or not. Species were assigned 1 based on either presence of alkaloid bands in the TLC plates, or from data in Daly et al. (1987). Species for which no alkaloids were detected using TLC, or that were reported by Daly and colleagues to lack alkaloids, were assigned 0. Three species warrant explanation. *Allobates femoralis* was reported to have trace alkaloids in one individual out of six populations (Daly et al. 1987); our TLC analysis detected no alkaloids from 15 skins from one locality; this species was scored as 0. Because *A. zaparo* displayed only trace alkaloid bands, we scored it as 0. *Epipedobates tricolor* was assigned 1 based on data from Daly et al. (1987) for *E. anthonyi*.

Comparative Method Analyses Using Independent Contrasts. Statistical analyses employed JMP (SAS Institute Inc. 2000). Niche breadth variables (NBNUM and NBVOL) were log-transformed; %ANTSNUM, %ANTSVOL, and %INDANTS were arcsine-transformed as appropriate for percentages (Sokal and Rohlf 1981). All

transformations improved the distributional properties of the data. All subsequent references to these variables refer to transformed data.

Our statistical analyses were designed to answer two questions: Are measures of diet specificity correlated with phylogenetic divergence (as measured by DNA sequences)? And, do diet variables predict the degree of toxicity? We used two general methods of analysis to examine the relationship between diet and toxicity while accounting for similarity due to common descent: conversion of variables to independent contrasts (Felsenstein 1985; Garland et al. 1992) and generalized least squares analysis of transformed variables (Martins and Hansen 1997; Pagel 1997). Both methods require a model phylogeny with branch lengths; this was taken from Santos et al. (2003). Santos et al. (2003) treated *Epipedobates anthonyi* under the name *E. tricolor*. Thus, our branch length data for *E. tricolor* refer to *E. anthonyi* if two distinct species are recognized. At the level of analysis used here, this phylogeny is unambiguous and well supported with respect to the placement of the focal species.

Sillén-Tullberg (1993) argued that the intensity of taxon sampling, and therefore the relative abundance of character states, can bias the results of comparative methods. This was discussed in the context of Maddison's (1990) concentrated changed test; however, the argument applies to all comparative methods. Although our sample of taxa was smaller than perhaps is optimal, this experimental design only makes the tests more conservative and less likely to reject the null hypothesis.

Principal component analyses (from correlation matrices) of the transformed variables were used to create new linear combinations of variables and thus to reduce dimensionality of the dataset. NBNUM and NBVOL were thus analyzed, and the score of each species on the first principal component were used to calculate independent contrasts for the new variable PCNB. Similarly, %ANTSNUM and %ANTSVOL were summarized using the first principal component (PC%ANTS). Also, the first principal component analysis of all six measures of diet specialization was calculated to summarize all diet variables (PCALL).

Using CAIC (Comparative Analysis by Independent Contrasts; Purvis and Rambaut 1994), we computed independent contrasts for Total Toxicity, the six measures of diet specificity, and the principal component scores. If diet data for a species were also available from Caldwell (1996), we averaged the two values. We plotted the absolute value of each set of standardized contrasts versus the standard deviation for that variable to check that each contrast had been adequately standardized (Garland et al. 1991; Garland et al. 1992). Hereafter, all mentions of contrasts refer to standardized contrasts. CAIC was also used to calculate nodal values for selected variables.

To assess the relationship between diet and toxicity, we used two approaches. First, correlation coefficients (forced through the origin) between Total Toxicity and each diet variable were calculated from the contrasts. Second, the contrasts of NUMPREY, %INDANTS, PC%ANTS, and PCNB were used as variables in a stepwise multiple regression (through the origin) to determine which measure of dietary specialization was

the best predictor of total toxicity. We used the contrasts from the principal components (PC%ANTS and PCNB) rather than contrasts from the %ANTSNUM, %ANTSVOL, NBNUM, and NBVOL because %ANTSNUM and %ANTSVOL are highly correlated, as are NBNUM and NBVOL. In this way we avoided potential problems with collinearity in predictor variables in the multiple regression analysis (Neter et al. 1996). The multiple regression analyses were repeated with and without the data for *E. tricolor*, because the contrast computed between *E. tricolor* and *E. boulengeri* was an extreme outlier due to the large difference in total toxicity and dietary specialization between the two species.

Comparative Method Analyses Using Generalized Least Squares. The GLS approach can be used to examine the degree to which trait variation is related to phylogeny as well as the degree to which two or more traits co-vary in a phylogenetic context (Freckleton et al. 2002). Both questions are special cases of a general method that integrates information about phylogeny and phenotypic variation and summarizes this as an "evolutionary regression coefficient" (Pagel 1993).

The matrix form of the GLS model is:

$$y = \beta X + e$$

A species trait is treated as the y-value to be predicted from a regression of that trait on some predictor variable X where ß is the regression coefficient. The maximum likelihood estimate of ß is found by

$$\beta = \left(X'V^{-1}X\right)^{-1}\left(X'V^{-1}Y\right)$$

where ß is the vector of regression coefficients, X is a matrix of predictor variables (see below), Y is the matrix of response variables, and V is the species-by-species variance-covariance matrix of the shared branch lengths of the tree.

The GLS model can be used to address the degree to which trait variation is related to phylogeny. The predictor X is a matrix of the sum of branch lengths from the root for each species. In this case, ß estimates the amount of change in y per unit change of evolutionary divergence (as measured by branch lengths indicating genetic divergence or time, for example). In this type of analysis, the variance in the observed trait y is explained by the regression equation and thus one can assess whether y co-varies with the amount of evolutionary divergence. In other words, are the observed values of y independent of the phylogeny, as would be expected if strong selection had obliterated phylogenetic information in the trait, or in contrast, do the values of y co-vary with the phylogeny, as expected if change in y follows a Brownian motion process?

X is a matrix of one or more species traits that are used to predict a matrix of species traits Y. Thus, one can estimate the evolutionary correlation between Y and one or more predictor variables, X. In this case, ß estimates the change in Y per unit change in X along branches in the tree. This type of analysis can produce a result essentially identical to that using Independent Contrasts under certain assumptions.

We used generalized least squares (GLS) as implemented in Continuous (Pagel 1997, 1999; see also Martins and Hansen 1997). To assess the degree to which a trait co-varies with phylogeny, Continuous provides a maximum likelihood estimate, termed λ, of the regression coefficient. The parameter λ measures the correlation between a trait and total divergence as 0 if the trait is completely independent of the phylogeny (no "phylogenetic effect") and 1 if the trait follows a Brownian motion process (complete covariance with the phylogeny). Continuous also provides a likelihood ratio test of the estimate of λ. For the variables used here, we tested the null hypothesis that $\lambda = 1$ (complete covariance with phylogeny) under a general assumption of Brownian motion evolution of the trait.

Using Continuous, we also estimated the correlation coefficient (taking phylogeny into account) between the two measures of toxicity and the diet variables. Continuous provides a likelihood-ratio test of the significance of the correlation coefficient. In this test, λ was estimated (rather than set to be 0 or 1) under both the null and alternative hypotheses, and the covariance between the two traits under the null hypothesis was constrained to be 0.

2.3 RESULTS

Diet Analyses. A total of 2,640 prey items in 46 prey categories were identified from 122 specimens of 9 species. Summaries of the diet analyses are presented in Figure 2.1, Table 2.1, and Table 2.3. PCNB (the first principal component derived from NBVOL and NBNUM) summarized 88% of the variance. PC%ANTS (for %ANTSVOL and %ANTSNUM) summarized 72.5% of the variance. PCALL (for all six measures of diet specialization) accounted for 69.5% of the total variance.

To visualize interspecific variation in diet specificity, histograms of percent of total prey volume for the 15 most abundant prey categories are presented with the phylogeny (Figure 2.1). The value for NBVOL (log-transformed niche breadth calculated by volume) follows the species name; this variable summarizes the distribution of prey item proportions in each category (Figure 2.1). Species with small values of NBVOL are interpreted as having a narrow diet. Ants (Formicidae) are usually the most abundant prey by volume in the species with small NBVOL values. Correspondingly, the histograms of specialists are largely skewed left (e.g. *Dendrobates auratus*). In contrast, species with large NBVOL values are interpreted as having a wide diet, and accordingly there are more, shorter bars in the histograms of these species (e.g. *Epipedobates boulengeri*).

Skin chemistry analyses. Of the nine species examined using thin layer chromatography, no alkaloids were detected in five species (*Allobates femoralis*, *Epipedobates boulengeri*, and all three *Colostethus*) (Table 2.2). Alkaloids were detected in all *E. bilinguis* and *E. parvulus* individuals assayed, and in 15 of 16 individuals of *E. hahneli*. All individuals of *A. zaparo* contained trace alkaloids: a very faint, single spot as opposed to dark multiple spotting or streaking.

Relationship between Traits and Phylogeny. We were able to reject the null hypothesis that $\lambda = 1$ for %INDANTS, NBNUM, %ANTSVOL, %ANTSNUM, PC%ANTS, PCALL, and Binary Toxicity, but not for NUMPREY, NBVOL, PCNB, or Total Toxicity (Table 2.4). Of the six basic diet measures, the smallest λs are seen in the three ant variables; the largest λs are seen in the three general diet measures. The diet variable that takes into account all six basic diet measures, PCALL, is uncorrelated with phylogeny.

Relationship between Diet and Toxicity. Stepwise multiple regression analyses of contrasts were used to determine which measure of diet specialization was the best predictor of Total Toxicity (Table 2.5). With the full dataset, PCNB was the best predictor of toxicity, followed by %INDANTS, and PC%ANTS. NUMPREY did not enter into the model. In the analyses excluding *Epipedobates tricolor*, PCNB was again found to be the best predictor, followed by NUMPREY, PC%ANTS and %INDANTS.

Based on the correlation coefficients calculated from contrasts, all diet-specialization measures were significantly ($P < 0.05$) correlated with Total Toxicity except NUMPREY

and %ANTSVOL (Figure 2.2). In the analyses excluding *Epipedobates tricolor*, PCNB, NBVOL, and PCALL were significantly ($P < 0.05$) correlated with Total Toxicity (Figure 2.2).

Using the GLS model and Total Toxicity from all 14 species, all dietary specialization measures were significantly ($P < 0.05$) correlated with Total Toxicity except %ANTSNUM and NUMPREY (Table 2.4). Using Binary Toxicity from all 14 species, all dietary specialization measures were significantly ($P < 0.05$) correlated with Binary Toxicity except NUMPREY (Table 2.4).

2.4 DISCUSSION

Diet and Phylogeny. How many times has a specialized diet evolved in dendrobatids? We divided the species evenly into two groups by NBVOL (other variables could be used), eight specialists and seven generalists, using a cutoff between *Phyllobates lugubris* (0.73) and *Epipedobates tricolor* (0.84). However, a characterization as generalist vs. specialist based on a continuous trait is arbitrary, and should be used for heuristic purposes rather than as an invariant character of a species. Mapping these two categories onto the phylogeny (Figure 2.1) indicates that a specialized diet evolved at least three times: in *Colostethus sauli*, in the clade of three *Epipedobates* (*hahneli*, *bilinguis*, and *parvulus*), and in the large clade of *Dendrobates* + *Phyllobates*.

Santos et al. (2003) postulated another origin of diet specialization in the brightly colored *Allobates zaparo* based on data from Almendáriz (1987). We found *A. zaparo* to have a much more generalized diet than reported by Almendáriz (1987). Additional data from the literature are consistent with our interpretation of three evolutionary origins of dietary specialization. Toft (1995) found the mean dietary niche breadth of three species of *Phyllobates* to be larger than that of the mean values for *Dendrobates* and most species of *Epipedobates*. She also reported the niche breadth of *Colostethus pratti* (included in her mean value for *Colostethus*) to be larger than that of *Phyllobates*, *Epipedobates*, and *Dendrobates*. The position of *C. pratti* in our tree is consistent with a plesiomorphic generalist diet in the clade containing *E. boulengeri*, *E. tricolor* (two more generalist species) as well as *E. parvulus*, *E. hahneli*, and *E. bilinguis*, species with smaller dietary niches.

The homoplasy in diet evolution that is evident in mapping NBVOL onto the phylogenetic tree is also evident in the degree to which diet variables co-vary with the phylogeny. The parameter λ ranges from 0, no phylogenetic effect, to 1, not distinguishable from a Brownian motion model (complete covariance with the phylogeny). Of the six diet variables, the smallest λs are seen in the three measures of ant specialization; the largest λs are seen in the three measures of general diet (Table 2.4). This pattern is consistent with natural selection promoting an evolutionary shift from more generalized to more ant-specialized diet in dendrobatids.

Correlation of Diet and Toxicity. Our results indicate that divergences from the ancestral, non-toxic state are significantly correlated with divergences in degree of dietary specialization in dendrobatids. In other words, we have verified the critical prediction of the diet-toxicity hypothesis in the context of multiple origins of aposematism: Independent origins of dietary specialization are correlated with the independent origins of toxicity. However, this correlation does not entail a one-to-one correspondence between aposematism and dietary specialization. For example, *Allobates zaparo* is brightly colored, but has only trace skin toxins and is a generalist (possibly due to Batesian mimicry; Darst work in progress). *Colostethus sauli* is cryptic and has no skin toxins, but is ant-specialized. *Epipedobates tricolor* is conspicuously colored, and its sister-species, *E. anthonyi* is known to have skin toxins, but *E. tricolor* exhibits a generalist diet. Low genetic divergence between *E. tricolor* and its cryptic sister taxon *C. machalilla* suggests a very recent origin of aposematism, which may contribute to the less specialized diet in *E. tricolor*. Also, *Epipedobates tricolor* (probably referable to *E. anthonyi*) contains the unique alkaloid epibatidine, for which the dietary source is unknown (Spande et al. 1992). Nonetheless, the association between dietary specialization and toxicity holds.

Certain diet variables are more strongly associated with toxicity than others. Using multiple regression, PCNB was the best predictor of toxicity in analyses with and without *Epipedobates tricolor*. Niche breadth quantifies each prey type eaten as a proportion of total observed prey types, and is, therefore, a general measure of diet. PCNB summarizes 88% of variation in NBVOL and NBNUM further generalizing this

measure. It is, therefore, not surprising PCNB was the best predictor of toxicity. In contrast, using the GLS models, %INDANTS was found to have the highest correlation coefficient with both Total Toxicity and Binary Toxicity; PCNB was in the top four correlation coefficients for both toxicity data sets. (It should be noted, however, that comparison of correlation coefficients alone does not account for partial correlations with other variables.) %INDANTS simply measures whether ants are at all a part of a species' diet; this suggests that the mere presence of ants is an important factor in sequestration of alkaloids.

Evolution of Sequestration in Dendrobatidae. De novo biosynthesis of toxins appears to be much more widespread in animals than sequestration, except for herbivorous insects (Termonia et al. 2002). Sequestration of pre-existing toxins is not necessarily cheaper or simpler than endogenous biosynthesis. An organism that actively accumulates toxic metabolites from another organism not only has to develop detoxification mechanisms (Duffey 1980), but also has to rely on diet for toxicity or must support toxin-producing symbionts (Mebs 2001).

The association of dietary specialization and sequestration of toxic defensive compounds is not novel to frogs. Lepidoptera (Brower 1958; Brower and Brower 1964; Alpin et al. 1968), Coleoptera (Eisner et al. 1962; Rowell-Rahier 1984), Orthoptera (von Euw et al. 1967), Hemiptera (Scudder and Duffey 1972; Braekman et al. 1982; Aliabadi et al. 2002); Hymenoptera (Schaffner et al. 1994), nudibranch mollusks (Thompson 1960; Edmunds 1966), reptiles (A. Savitzky, pers. comm.), and possibly birds (Dumbacher et

al. 1992, 2000) sequester defensive chemicals from their diet. However, the repeated association of these traits in the context of an explicit phylogeny has rarely been demonstrated.

Amphibian skin has two kinds of glands: 1) mucous glands, and 2) serous (or granular) glands, which are the "poison glands" of amphibians (Duellman and Trueb 1986). Neuwirth et al. (1979) suggested that serous glands are plesiomorphic among amphibians and their original function was probably other than poison synthesis, and, therefore, the glands were co-opted for toxin production and/or storage of sequestered compounds. The actual process of sequestration in poison frogs is unknown. In Lepidoptera, substances to be sequestered are (a) reabsorbed through the gut membrane (but not broken down), (b) transported into the hemolymph, and (c) deposited in particular sites of the body (Duffey 1980; Nishida 2002). Many sequestered chemicals are distributed non-randomly, concentrated in the peripheral integument and wings. In poison frogs, alkaloids are sequestered in the skin and are not found in the muscle or internal organs (Daly et al. 1994). Also, bitter taste is common in many naturally occurring toxins, including those in the skin of dendrobatids (Daly and Myers 1967; Nishida 2002). Peripheral distribution and bitter taste of toxins may have been important in facilitating the evolution of aposematism because the predators could directly sample tissues without inflicting lethal injuries.

Sequestration apparently has evolved independently in three lineages of frogs: Dendrobatidae, *Mantella* of the Madagascan Mantellidae, and possibly the South

American bufonid *Melanophryniscus* (Garraffo et al. 1993a, 1993b; Daly et al. 1996, 1997). Six classes of frog alkaloids are known to come from ants, one from beetles, and one from a millipede (Daly et al. 1997, 1999, 2002; Jones et al. 1999; Saporito et al. 2003). However, as far as is known, dendrobatid frogs are unique among vertebrates in their recurring evolutionary association of toxicity and dietary specialization. The recurrence of correlated instances of diet and toxicity in Dendrobatidae suggests either the presence of an ancestral sequestration mechanism, or that the evolution of a sequestration mechanism may not be particularly complex or difficult to attain (Caldwell 1996; Summers and Clough 2001; Santos et al. 2003; Summers 2003). But, if all dendrobatids have the capacity to sequester toxins, why do all species not do it? Daly (1998) suggested that an uptake system (or mechanism for eliminating toxins) is primitive and merely over-expressed as sequestration in frogs that accumulate alkaloids. Daly et al. (1994) found that *Colostethus talamancae* and *C. inguinalis* did not accumulate alkaloids into their skin after being fed with alkaloid-dusted fruit flies for five weeks; an identical feeding regimen did result in accumulation of alkaloids in *Dendrobates auratus* and *Phyllobates bicolor*. No adverse effects were reported in those frogs that did not accumulate the alkaloids suggesting a mechanism for eliminating toxins. Also, *Colostethus sauli* does not have skin toxins, but has a small niche breadth, and ants are significant prey (Table 2.1; Figure 2.1). These data support Daly's (1998) suggestion that the toxin elimination system itself is primitive, and that the ability to sequester the toxins in the skin may be an over-expression of this uptake system.

Diurnality and Terrestriality as Possible Innovations. Myers et al. (1991) postulated that diurnal and terrestrial habits evolved early in dendrobatid phylogeny based on the nocturnal and aquatic habits of *Aromobates nocturnus,* which was hypothesized to be the sister species of all other dendrobatids. Given this, diurnality and the transition from aquatic (*A. nocturnus*), to riparian (various species of *Colostethus*), and finally to terrestrial (all other dendrobatids) habits has preceded the origins of dietary specialization. The evolution of diurnality and/or shift to terrestriality may be the innovation(s) that contributed to the parallel evolution of toxicity-mediated by predation (Toft 1981; Vences et al. 1997/98). This shift may have exposed frogs to new food sources, assuming the diurnal leaf-litter arthropod community is different than the nocturnal arthropod community, and/or that new prey species were encountered in the move to terrestriality. If a small degree of toxicity (possibly acquired as a by-product of a mechanism for eliminating toxins) conferred at least some protection from predation, then natural selection exerted by predators would continually favor increasing toxicity, thereby promoting greater dietary specialization and a more efficient sequestration mechanism. Moreover, at least three species of *Dendrobates* have evolved a more efficient sequestration mechanism that converts a pumiliotoxin to a much more toxic allopumiliotoxin (Daly et al. 2003).

Once toxicity has evolved, any signal that identifies the frog as poisonous, such as bright coloration, would be favored. Coupled with predator learning, warning coloration would allow the aposematic individuals greater freedom to search out particular prey, reinforcing dietary specialization and increasing toxicity. *Dendrobates* forage constantly,

searching actively for prey as compared to cryptic, less diet-specialized *Colostethus*, which are quick to hide (Daly et al. 1971; Toft 1980). Toxic dendrobatids also tend to be more visibly mobile, and less flight-reactive; but, counter-examples do exist and these behaviors should be studied in more detail.

Criticisms of Comparative Methods. Some researchers have questioned the validity of comparative approaches for the study of evolution of aposematism in dendrobatids. Daly et al. (2002) criticized Summers and Clough's (2001) use of quantified toxicity data, arguing that use of computed toxicity values in comparative methods assumes that the availability of arthropod prey, and thus the profiles of alkaloids, have remained constant in evolutionary time. Daly and colleagues also pointed out that profiles of alkaloids and color pattern can vary microgeographically (Daly and Myers 1967; Daly et al. 2002). Particularly, the variability in alkaloid profiles in a single species (e.g. *Dendrobates pumilio;* Daly and Myers 1967) and the fact that this profile is a summation of alkaloids sequestered during the frog's life have been seen by critics as proximate factors that confound the meaningfulness of comparative analyses.

The perspective of Daly et al. (2002) regarding comparative methods seems to emerge from a proximal, functionalist approach to explaining historical patterns. According to this viewpoint, current interactions would be best explained by immediate ecological factors, ignoring phyletic constraint (Gould and Lewontin 1979). We argue, in contrast, that an historical perspective suggests that the apparent universality of toxicity and dietary specialization in the large clade of *Dendrobates* + *Phyllobates* (42 species:

Frost 2002) indicates that toxicity and a shift to specialized diet were present in its common ancestor. Ecology, in this case, diet, is inextricably linked to intrinsic organismal traits, such as behavior, physiology and morphology, all of which have a genetic basis, are heritable and therefore shaped by evolutionary history. However, application of comparative methods does not depend on the trait being under direct genetic control nor does it require that traits lack intraspecific variation (Freckelton et al. 2002). Traits may be correlated by common ancestry or ecological similarities of closely related species (Freckleton et al. 2002). Whether the traits have a genetic basis or not, comparative methods take into account the covariance structure of species' traits imposed by evolutionary history (Felsenstein 1985; Harvey and Pagel 1991).

2.5 CONCLUSIONS

Earlier studies demonstrated a correlation between independent origins of bright coloration and toxicity in poison frogs (Santos et al. 2003). Our study quantified diet in a select sample of species from across the dendrobatid phylogeny and demonstrated an association between diet and toxicity. Specifically, independent evolutionary origins of dietary specialization are correlated with the origins of toxicity (and, therefore, bright coloration). From these associations we infer the importance of ecological shifts in diet to the acquisition of new defenses against predation. The appearances of toxic skin alkaloids may have promoted the development of aposematic coloration as a reinforcement of this defense mechanism. This series of ecological shifts and

evolutionary transitions in dendrobatids may have been contingent on the presence of an ancestral mechanism for elimination of toxins and an ecological shift to diurnal activity and terrestrial (rather than aquatic or riparian) habits.

Table 2.1: Sample sizes, number of prey categories, mean number of prey per individual, frequency of individual frogs of that consumed ants, number of ants as a percentage of total diet per individual, and volume of ants as a percentage of total diet per individual for dendrobatid frogs examined in this study. Means are ± standard error. Niche breadths were calculated for number and volume of prey.

Species	Source	N	No. prey per individual (mean)	Proport. of individuals that ate ants	Percentage ants in diet (number)	Percentage ants in diet (volume)	Niche breadth (number)	Niche breadth (volume)
Allobates femoralis		15	4.3 ± 0.8	80%	35%	12%	6.06	7.25
Allobates femoralis	Caldwell	18	7.5 ± 1.4	61%	24%	6%	10.63	12.01
Allobates zaparo		20	9.7 ± 1.4	65%	26%	4%	8.31	8.14
Colostethus bocagei		22	6.0 ± 0.8	64%	21%	9%	3.07	8.47
Colostethus insperatus		12	6.7 ± 1.1	73%	39%	21%	5.09	7.90
Colostethus sauli		9	5.3 ± 1.6	75%	60%	53%	2.71	3.09
Colostethus talamancae	Caldwell	19	13.9 ± 2.3	74%	21%	16%	7.18	11.94

Dendrobates auratus	Caldwell	23	186.5 ± 24.0	100%	63%	73%	2.03	1.86
Dendrobates pumilio	Caldwell	33	84.4 ± 9.3	100%	27%	50%	2.25	2.75
Dendrobates ventrimaculatus	Caldwell	5	70.8 ± 19.4	100%	33%	68%	3.14	1.97
Epipedobates bilinguis		24	61.0 ± 6.5	100%	67%	37%	2.06	3.63
Epipedobates bilinguis	Caldwell	32	47.7 ± 3.6	100%	56.8%	37%	2.88	3.88
Epipedobates boulengeri	Caldwell	32	28.2 ± 3.0	25%	1%	4%	5.38	12.72
Epipedobates hahneli		11	23.6 ± 8.6	80%	75%	15%	1.77	4.02
Epipedobates parvulus		2	37.5 ± 15.5	100%	89%	39%	1.25	3.03
Epipedobates tricolor		7	7.6 ± 1.4	71%	50%	17%	3.18	6.85
Phyllobates lugubris	Caldwell	12	29.4 ± 8.3	100%	41%	35%	3.91	5.38

Note: *E. bilinguis* and *C. bocagei* each had outliers in number of prey items per individual (281 "dipteran larvae" and 134 "eggs," respectively); these outliers were removed in calculation of average and standard deviation.

Table 2.2. Thin layer chromatography results are reported as positive (P): presence of alkaloids in skin extract, negative (N): no alkaloids, or trace (T): possibly very small amount of alkaloids; diversity, quantity and lethality of species as taken from Summers and Clough (2001), compiled from Daly et al. (1987); and our composite scores of Total Toxicity and Binary Toxicity.

Species	No. of skins	TLC	Diversity	Quantity	Lethality	Total Toxicity	Binary Toxicity
Allobates femoralis	15	N	0.17	0.17	1	1.2	0
Allobates zaparo	20	T	–	–	–	2.0	0
Colostethus bocagei	22	N	–	–	–	0	0
Colostethus insperatus	12	N	–	–	–	0	0
Colostethus sauli	10	N	–	–	–	0	0
Colostethus talamancae	–	–	0	0	0	0	0
Dendrobates auratus	–	–	16.8	2.56	2	6.2	1
Dendrobates pumilio	–	–	16.0	2.25	2	5.9	1

Dendrobates ventrimaculatus	–	–	12.5	2.0	2	5.3	1
Epipedobates bilinguis	24	P	6.0	1.5	1	3.1	1
Epipedobates boulengeri	20	N	–	–	–	0	0
Epipedobates hahneli	16	P	6.0	2.0	1	3.6	1
Epipedobates parvulus	2	P	–	–	–	3.4	1
Epipedobates tricolor	–	–	2.0	1	1	2.2	1
Phyllobates lugubris	–	–	1.7	0.67	3	3.8	1

Note: Alkaloids were detected in 15 out of 16 *E. hahneli* skin extracts.

Table 2.3. Numeric and volumetric data of prey items in the gastrointestinal tracts of dendrobatids. Data are presented in alphabetic order of species and prey categories.

	Prey category	Number	% number	Volume (mm^3)	% volume
Allobates femoralis (*n*=15)					
	Araneae	3	5.00	22.44	14.04
	Chalcidoidea	2	3.33	0.04	0.03
	Chilopoda	2	3.33	43.89	27.47
	Coleoptera	5	8.33	8.47	5.30
	Collembola	2	3.33	0.53	0.33
	Diplopoda	1	1.67	1.94	1.21
	Diptera	5	8.33	8.50	5.32
	Elateridae	1	1.67	10.75	6.73
	Formicidae	21	35.00	18.87	11.81
	Hymenoptera	6	10.00	11.76	7.36
	Isoptera	1	1.67	3.33	2.08
	Larvae unidentified	2	3.33	9.74	6.10
	Material Plant	6	10.00	1.06	0.66

	Prostigmata	1	1.67	0.03	0.02
	Reduviidae	1	1.67	11.29	7.06
	Scarabaeidae	1	1.67	7.14	4.47
	Total	60	100.00	159.78	100.00
Allobates zaparo (*n*=20)					
	Acari	4	2.22	0.22	0.04
	Aphididae	2	1.11	1.88	0.30
	Araneae	3	1.67	7.30	1.18
	Chalcidoidea	2	1.11	0.34	0.06
	Chilopoda	2	1.11	56.52	9.17
	Coleoptera	18	10.00	65.62	10.64
	Collembola	8	4.44	1.72	0.28
	Curculionidae	1	0.56	4.81	0.78
	Diplopoda	2	1.11	1.29	0.21
	Diptera	6	3.33	1.25	0.20
	Formicidae	47	26.11	26.30	4.26

Gastropoda	1	0.56	7.79	1.26
Gryllidae	1	0.56	7.94	1.29
Hemiptera	3	1.67	1.84	0.30
Hymenoptera	7	3.89	33.73	5.47
Isoptera	30	16.67	144.09	23.37
Larvae unidentified	2	1.11	0.84	0.14
Larvae Coleoptera	3	1.67	81.44	13.21
Larvae Diptera	6	3.33	1.93	0.31
Larvae Lampyridae	3	1.67	90.61	14.69
Material Plant	12	6.67	0.13	0.02
Nitidulidae	4	2.22	22.48	3.65
Orthoptera	1	0.56	9.39	1.52
Pentatomidae	1	0.56	2.13	0.35
Reduviidae	2	1.11	3.59	0.58
Scolytidae	5	2.78	14.65	2.38
Staphylinidae	4	2.22	26.82	4.35
Total	180	100.00	616.64	100.00

Colostethus bocagei (*n*=22)

Acari	2	0.83	0.04	0.01
Araneae	4	1.66	107.85	28.19
Chilopoda	1	0.41	0.13	0.03
Coccinellidae	1	0.41	0.03	0.01
Coleoptera	8	3.32	32.48	8.49
Curculionidae	4	1.66	10.34	2.70
Decapoda	1	0.41	27.44	7.17
Diplopoda	1	0.41	0.29	0.08
Diptera	4	1.66	1.46	0.38
Eggs?	127	52.70	5.92	1.55
Formicidae	50	20.75	32.29	8.44
Hemiptera	11	4.56	16.78	4.39
Homoptera	2	0.83	6.29	1.64
Hymenoptera	5	2.07	14.26	3.73
Isoptera	2	0.83	12.41	3.24

Larvae unidentified	3	1.24	10.17	2.66
Larvae Coleoptera	2	0.83	6.24	1.63
Larvae Lampyridae	1	0.41	24.08	6.29
Larvae Lepidoptera	1	0.41	14.61	3.82
Membracidae	1	0.41	9.28	2.43
Material Plant	4	1.66	1.84	0.48
Nitidulidae	1	0.41	1.36	0.35
Scarabaeidae	1	0.41	8.26	2.16
Scolytidae	1	0.41	11.06	2.89
Staphylinidae	1	0.41	0.27	0.07
Tenebrionidae	2	0.83	27.37	7.16
Total	241	100.00	382.55	100.00

Colostethus insperatus (*n*=12)

Acari	9	12.16	0.84	6.06
Araneae	7	9.46	2.32	16.67
Chalcidoidea	1	1.35	0.06	0.42

Coleoptera	1	1.35	0.03	0.23
Collembola	5	6.76	0.20	1.43
Curculionidae	2	2.70	0.77	5.56
Diplopoda	1	1.35	1.34	9.61
Diptera	1	1.35	0.03	0.24
Formicidae	29	39.19	2.93	21.00
Homoptera	3	4.05	1.25	9.00
Hymenoptera	4	5.41	1.92	13.77
Larvae unidentified	6	8.11	1.40	10.05
Material Plant	3	4.05	0.14	1.02
Scolytidae	1	1.35	0.37	2.66
Staphylinidae	1	1.35	0.32	2.28
Total	74	100.00	13.93	100.00

Colostethus sauli (*n*=9)

Araneae	1	2.38	0.56	1.13
Cicadellidae	3	7.14	2.20	4.39

Cucujidae	1	2.38	0.34	0.69
Elateridae	1	2.38	2.16	4.33
Formicidae	25	59.52	26.26	52.50
Hemiptera	1	2.38	2.61	5.22
Homoptera	1	2.38	0.03	0.05
Hymenoptera	1	2.38	1.24	2.48
Larvae unidentified	2	4.76	1.27	2.53
Larvae Lepidoptera	1	2.38	0.08	0.15
Membracidae	1	2.38	2.64	5.28
Material Plant	1	2.38	0.43	0.85
Orthoptera	1	2.38	0.00	0.00
Scolytidae	1	2.38	0.53	1.06
Thyreocoridae	1	2.38	9.68	19.34
Total	42	100.00	50.02	100.00

Epipedobates bilinguis (*n*=24)

Acari	105	6.26	9.90	3.30

Araneae	7	0.42	1.56	0.52
Chilopoda	1	0.06	0.01	0.00
Coleoptera	7	0.42	18.25	6.08
Collembola	21	1.25	2.03	0.68
Diplopoda	3	0.18	1.30	0.43
Diptera	1	0.06	0.01	0.00
Formicidae	1127	67.24	109.91	36.62
Homoptera	9	0.54	5.44	1.81
Hymenoptera	9	0.54	1.14	0.38
Isoptera	61	3.64	109.47	36.47
Larvae unidentified	15	0.89	8.34	2.78
Larvae Diptera	284	16.95	9.93	3.31
Larvae Lampyridae	1	0.06	5.30	1.77
Larvae Neuroptera	1	0.06	0.65	0.22
Larvae Orthoptera	1	0.06	0.07	0.02
Miridae	1	0.06	0.78	0.26
Material Plant	8	0.48	3.31	1.10

	Nitidulidae	1	0.06	0.08	0.03
	Pselaphidae	1	0.06	0.25	0.08
	Scolytidae	10	0.60	9.71	3.23
	Tenebrionidae	2	0.12	2.68	0.89
	Total	1676	100.00	300.12	100.00
Epipedobates hahneli (*n*=11)					
	Acari	14	6.11	2.54	2.17
	Aphididae	1	0.44	0.50	0.42
	Araneae	2	0.87	0.92	0.79
	Blattaria	1	0.44	10.77	9.23
	Chilopoda	2	0.87	0.21	0.18
	Coleoptera	8	3.49	49.08	42.03
	Collembola	1	0.44	0.06	0.05
	Curculionidae	1	0.44	0.91	0.78
	Diptera	1	0.44	0.00	0.00
	Formicidae	171	74.67	17.09	14.64

Homoptera	3	1.31	1.18	1.01
Hymenoptera	1	0.44	0.11	0.09
Isoptera	7	3.06	22.79	19.51
Larvae unidentified	1	0.44	0.00	0.00
Larvae Coleoptera	1	0.44	4.48	3.83
Larvae Lepidoptera	3	1.31	4.69	4.02
Material Plant	6	2.62	0.06	0.05
Scolytidae	1	0.44	0.95	0.81
Staphylinidae	4	1.75	0.44	0.37
Total	229	100.00	116.77	100.00

Epipedobates parvulus (*n*=2)

Acari	3	4.00	0.62	3.38
Curculionidae	1	1.33	6.89	37.77
Formicidae	67	89.33	7.17	39.36
Hymenoptera	1	1.33	0.00	0.00
Larvae unidentified	2	2.67	3.19	17.48

	Tetranychidae	1	1.33	0.36	2.00
	Total	75	100.00	18.23	100.00
Epipedobates tricolor (n=7)					
	Acari	12	24.50	1.31	13.73
	Brentidae	1	2.04	0.62	6.54
	Coleoptera	2	4.08	0.70	7.33
	Diptera	1	2.04	0.04	0.44
	Formicidae	24	48.98	1.57	16.45
	Hemiptera	1	2.04	0.33	3.44
	Hymenoptera	5	10.20	1.63	17.09
	Larva	1	2.04	1.97	20.67
	Larva Lepidoptera	1	2.04	1.25	13.09
	Pupa	1	2.04	0.12	1.22
	Total	49	100.00	9.53	100.00

Table 2.4. Lambdas and correlations coefficients as calculated using the GLS model. Both Total Toxicity and Binary Toxicity scores were used.

			Total Toxicity		Binary Toxicity	
Dietary specialization measure	λ	Probability (H_0: $\lambda = 1.0$)	Correlation coefficient	Probability (H_0: $r = 0$)	Correlation coefficient	Probability (H_0: $r = 0$)
NUMPREY	1.000	1.000	0.517	0.077	–0.292	0.309
%INDANTS	0.647	0.011	0.717	0.001	0.766	< 0.001
NBVOL	0.730	0.078	– 0.690	0.002	– 0.607	0.009
NBNUM	0.785	0.046	– 0.567	0.018	– 0.603	0.009
%ANTSVOL	0.534	0.028	0.524	0.035	0.502	0.038
%ANTSNUM	0.000	< 0.001	0.441	0.075	0.589	0.013
PCNB	0.780	0.059	– 0.682	0.002	– 0.651	0.004
PC%ANTS	0.099	< 0.001	0.538	0.024	0.620	0.007

PCALL	0.656	0.018	0.707	0.001	0.707	0.001

Note: Total toxicity: $\lambda = 0.899$; p (H_0: $\lambda = 1.0$) = 0.159; Binary toxicity: $\lambda = 0.631$; p (H_0: $\lambda = 1.0$) = 0.001

Table 2.5. Order of entry and F-to-enter scores for stepwise regression for Total Toxicity contrasts as predicted by dietary specialization contrasts with and without data from *Epipedobates tricolor*.

	with *E. tricolor*		without *E. tricolor*	
Measure of dietary specialization	Order of entry	F to enter	Order of entry	F to enter
PCNB (First principal component of niche breath by volume and niche breadth by number)	1	8.22	1	9.86
%INDANTS (Proportion of individuals that ate ants)	2	6.04	4	3.39
PC%ANTS (First principal component of % ants in the diet by volume and % ants in the diet by number)	3	4.02	3	6.36
NUMPREY (Number of prey per individual)	4*	0.03	2	1.68

*The entry probability of NUMPREY was not significant.

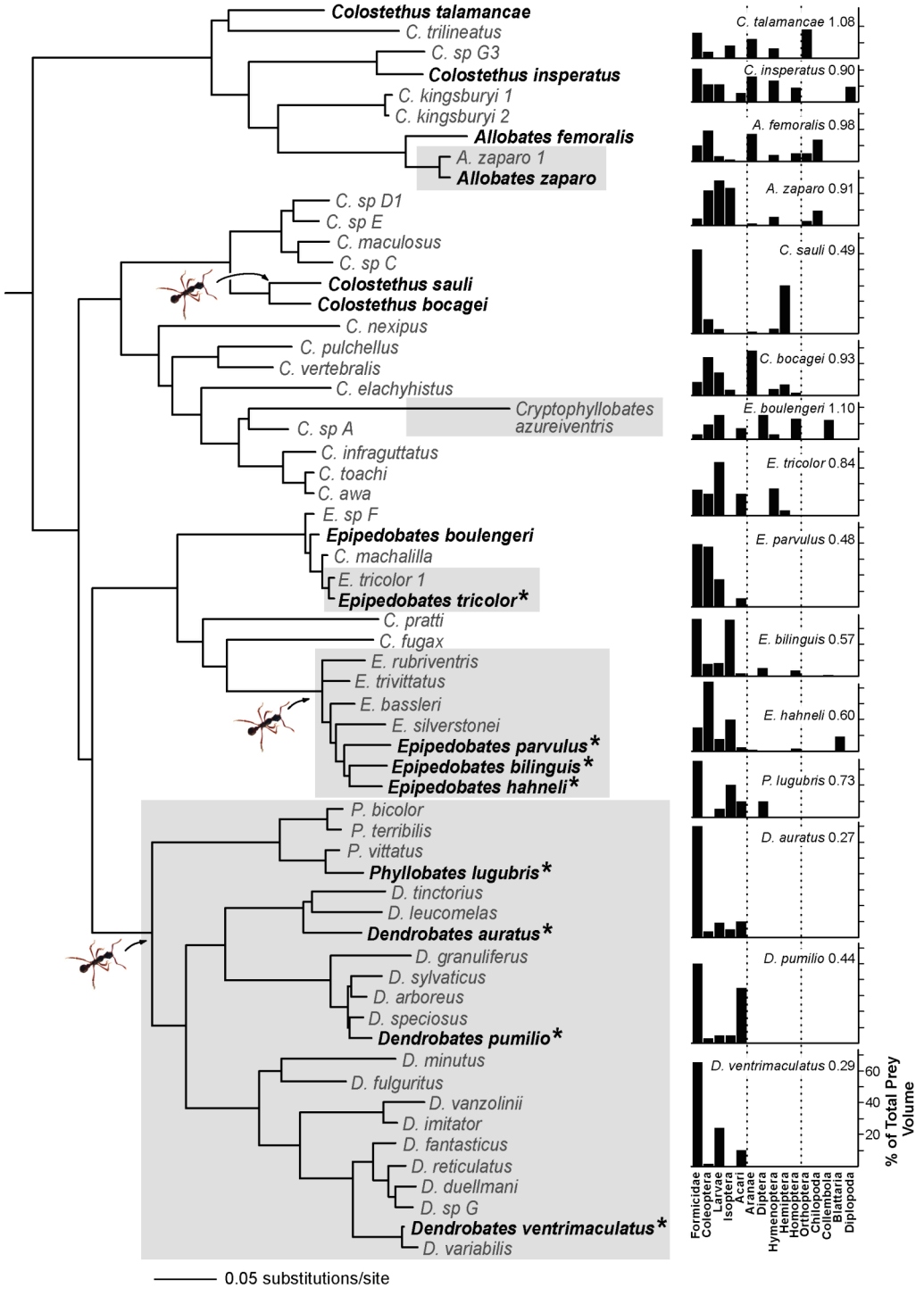

Figure 2.1. Maximum liklihood phylogeny of Dendrobatidae from Santos et al. (2003). The species names in bold are those examined in the present study. The gray boxes

represent conspicuous species. We assigned presence/absence toxicity scores to species in bold; asterisks denote presence of alkaloids. Ant icons indicate evolutionary origins of specialized diets. Histograms depict percentage of total prey volume for the fifteen most abundant prey categories. Values of NBVOL (log-transformed niche breadth by volume) are given for comparison on the histograms immediately following each species name.

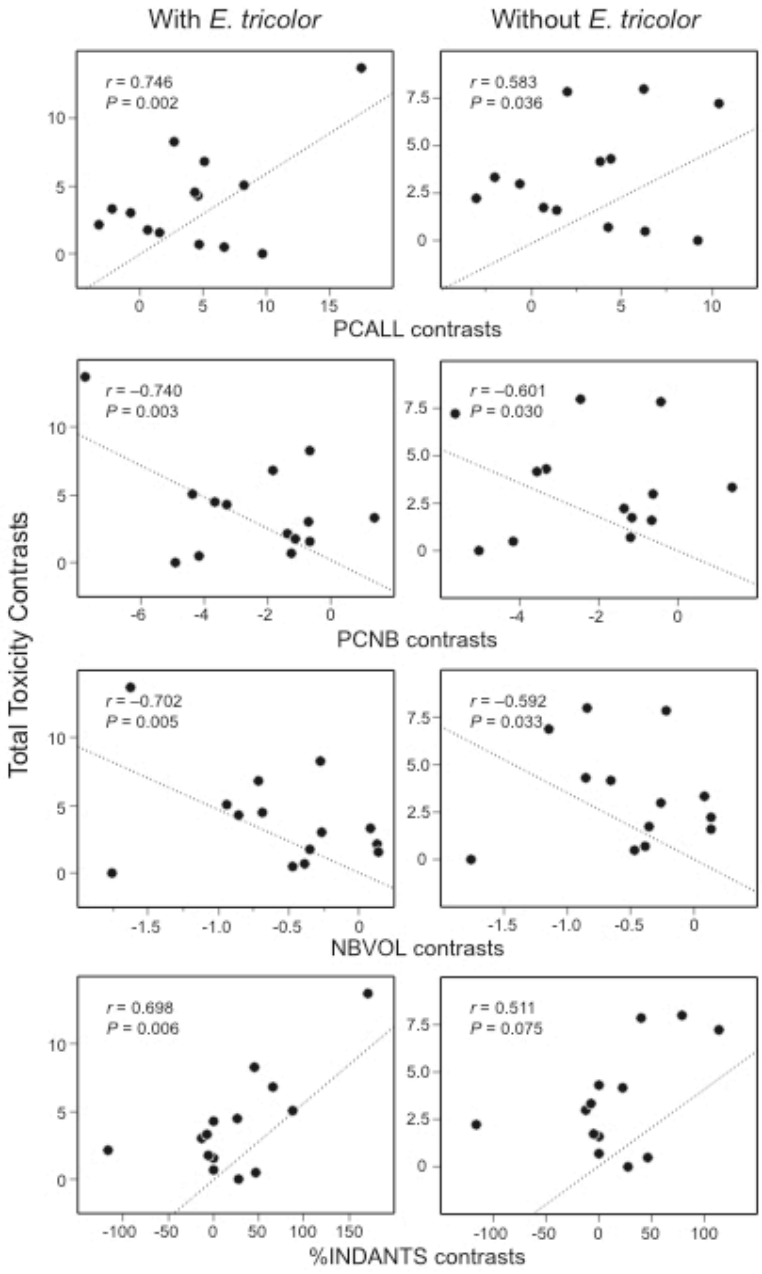

Figure 2.2. Bivariate plots of Total Toxicity contrasts against contrasts of diet variables. Correlation coefficients (*r* values) were calculated with regression through the origin.

References

Aliabadi, A., Renwick, J. A. A., & Whitman, D. W. 2002. Sequestration of glucosinolates by harlequin bug *Murgantia histrionica*. Journal of Chemical Ecology 28: 1749–1762.

Almendáriz, A. 1987. Contribucion al conocimiento de la herpetofauna centroiental ecuatoriana. Revista Politécnica, Quito 12: 144–177.

Alpin, R. T., Benn, M. H., & Rothschild, M. 1968. Poisonous alkaloids in the body tissues of the cinnabar moth (*Callimorpha jacobaeae* L.) Nature 219: 747–748.

Becerra, J. X. 1997. Insects on plants: macroevolutionary chemical trends in host use. Science 276: 253–256.

Becerra, J. X. & Venable, D. L. 1999. Macro-evolution of insect-plant associations: the relevance of host biogeography to host affiliation. Proceedings of the National Academy of Sciences USA 96: 12626–12631.

Berenbaum, M. 1983. Coumarins and caterpillars: a case for coevolution. Evolution 37: 163–179.

Braekman, J. C., Daloze, D., & Pasteels, J. M. 1982. Cyanogenic and other plant glucosides in a New Guinea bug *Leptocoris isolata*: possible precursors in its host plant. Biochemical Systematics and Ecology 10: 355–364.

Brower, L. P. 1958. Bird predation and food-plant specificity in closely related procryptic insects. American Naturalist 92: 183–187.

Brower, L. P. & Brower, J. V. Z. 1964. Birds, butterflies and plant poisons: a study in ecological chemistry. Zoologica 49: 137–159.

Caldwell, J. P. 1996. The evolution of myrmecophagy and its correlates in poison frogs (family Dendrobatidae). Journal of Zoology, London 240: 75–101.

Clough, M. & Summers, K. 2000. Phylogenetic systematics and biogeography of the poison frogs: evidence from mitochondrial DNA sequences. Biological Journal of the Linnean Society 70: 515–540.

Cott, H. B. 1940. Adaptive coloration in animals. Methuen, London.

Daly, J. W. 1998. Thirty years of discovering arthropod alkaloids in amphibian skin. Journal of Natural Products 61: 162–172.

Daly, J. W. & Myers, C. W. 1967. Toxicity of Panamanian poison frogs (*Dendrobates*): some biological and chemical aspects. Science 156: 970–973.

Daly, J. W., Myers, C. W., & Whittaker, N. 1987. Further classification of skin alkaloids from Neotropical poison frogs (Dendrobatidae), with a general survey of toxic/noxious substances in the Amphibia. Toxicon 25: 1023–1095.

Daly, J. W., Myers, C. W., Warnick, J. E., & Albuquerque, E. X. 1980. Levels of batrachotoxin and lack of sensitivity to its action in poison-dart frogs (*Phyllobates*). Science 208: 1383–1385.

Daly, J. W., Garraffo, H. M., & Spande, T. F. 1999. Alkaloids from amphibian skin. Pages 1–161 *in* S. W. Pelletier, ed. Alkaloids: Chemical and Biological Perspectives (Vol. 13). Pergamon, New York.

Daly, J. W., Garraffo, H. M., Hall, G. S. E., & Cover Jr., J. F. 1997. Absence of skin alkaloids in captive-raised Madagascan mantelline frogs (*Mantella*) and sequestration of dietary alkaloids. Toxicon 35: 1131–1135.

Daly, J. W., Garraffo, H. M., Poonam, J., Spande, T. F., Snelling, R. R., Jaramillo, C., & Rand, A. S. 2000. Arthropod-frog connection: decahydroquinoline and pyrrolizidine alkaloids common to microsympatric myrmicine ants and dendrobatid frogs. Journal of Chemical Ecology 26: 73–85.

Daly, J. W., Garraffo, H. M. Spande, T. F., Jaramillo, C., & Rand, A. S. 1994. Dietary source for skin alkaloids of poison frogs (Dendrobatidae)? Journal of Chemical Ecology 20: 943–955.

Daly, J. W., Garraffo, H. M., Spande, T. F., Clark, V. C., Ma, J., Ziffer, H., & Cover Jr., J. F. 2003. Evidence for an enantioselective pumiliotoxin 7-hydroxlase in dendrobatid poison frogs of the genus *Dendrobates*. Proceedings of the National Academy of Sciences USA 100: 11092–11097.

Daly, J. W., Karle, I., Myers, C. W., Tokuyama, T., Waters, J. A., & Witkop, B. 1971. Histrionicotoxins: Roentgen-ray analysis of the novel allenic and acetylenic

spiroalkaloids isolated from a Colombian frog, *Dendrobates histrionicus*. Proceedings of the National Academy of Sciences USA 68: 1870–1875.

Daly, J. W., Andriamaharavo, N. R., Andriantsiferana, M., & Myers, C. W. 1996. Madagascar poison frogs (*Mantella*) and their skin alkaloids. American Museum Novitates 3177: 1–34.

Daly, J. W., Kaneko, T., Wilham, J., Garraffo, H. M., Spande, T. F., Espinosa, A., Donnelly, & M. A. 2002. Bioactive alkaloids of frog skin: combinatorial bioprospecting reveals that pumiliotoxins have an arthropod source. Proceedings of the National Academy of Sciences USA 99: 13996–14001.

Darst, C R. & Cannatella, D. C. 2004. Novel relationships among hyloid frogs inferred from 12S and 16S mitochondrial DNA sequences. Molecular Phylogenetics and Evolution. 31: 462–475

Denno, R. F. S., Larsson, S., & Olmstead, K. L. 1990. Role of enemy-free space and plant quality in host-plant selection by willow beetles. Ecology 71: 124–137.

Dobler, S. 2001. Evolutionary aspects of defense by recycled plant compounds in herbivorous insects. Basic Applied Ecology 2: 15–26.

Donnelly, M. A. 1991. Feeding patterns of the strawberry poison frog, *Dendrobates pumilio* (Anura: Dendrobatidae). Copeia 1991: 723–730.

Duellman, W. E. & Trueb, L. 1986. Biology of Amphibians. John Hopkins University Press, Baltimore, Maryland.

Duffey, S. S. 1980. Sequestration of plant natural products by insects. Annual Review of Entomology 25: 447–477.

Dumbacher, J. P., Beehler, J. W., Spande, T. F., Garraffo, H. M., & Daly, J. W. 1992. Homobatrachotoxin in the genus *Pitohui*: chemical defense in birds? Science 258: 799–801.

Dumbacher, J. P., Spande, T. F., & Daly, J. W. 2000. Batrachotoxin alkaloids from passerine birds: a second toxic bird genus (*Ifrita kowaldi*) from New Guinea. Proceedings of the National Academy of Sciences USA 97: 12970–12975.

Dunhan, A. E. 1983. Realized niche overlap: resource abundance and intensity of interspecific competition. Pages 261-280 *in* R. B. Huey, E. R. Pianka, and T. Shoener, eds. Lizard Ecology: Studies of a Model Organism. Harvard University Press, England.

Edmunds, M. 1966. Protective mechanisms in the Eolidacea (Mollusca: Nudibranchia). Journal of the Linnean Society (Zoology) 46: 27–71.

Edmunds, M. 1974. Defense in Animals. Longman, Harlow.

Edmunds, M. 1987. Color in opisthobranchs. American Malacological Bulletin 5: 185–196.

Ehrlich, P. R. & Raven, P. H. 1964. Butterflies and plants: a study in coevolution. Evolution 18: 586–608.

Eisner, T. 1970. Chemical defense against predation in arthropods. Pages 157–217 *in* E. Sondheimer and J. B. Simeone, eds. Chemical Ecology. Academic Press, New York.

Eisner, T., Kafatos, F. C.,& Linsley, E. G. 1962. Lycid predation by mimetic adult Cerambycidae (Coleoptera). Evolution 16: 316–324.

Farrell, B. & Mitter, C. 1990. Phylogenies of insect/plant interactions: have Phyllobrotica leaf beetles (Chrysomelidae) and the Lamiales diversifies in parallel? Evolution 44: 1389–1403.

Feeny, P. P. 1987. The roles of plant chemistry in association between swallowtail butterflies and their host plants. Pages 53–61 *in* Proceedings of the 6th International Symposium on Insect-plant relations, Junk Publications, Dordrecht.

Felsenstein, J. 1985. Phylogenies and the comparative method. American Naturalist 125: 1–15.

Freckleton, R. P, Harvey, P. H. & Pagel, M. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. American Naturalist 160: 712–726.

Fritz, G., Rand, A. S., & dePamphilis, C. W. 1981. The aposematically colored frog, *Dendrobates pumilio*, is distasteful to the large, predatory ant, *Paraponera clavata*. Biotropica 13: 158–159.

Frost, D. R. 2002. Amphibian species of the world: an online reference. V2.21 (15 July 2002) Electronic database available at research.amnh.org/herpetology/amphibia/index.html

Futuyma, D. J. 1983. Evolutionary interactions among herbivorous insects and plants. Pages 207–231 *in* D. J. Futuyma and M. Slatkin, eds. Patterns and Processes in the Evolution of Life. Springer-Verlag, Berlin.

Garland, T. Jr., Harvey, P. H., & Ives, A. R. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. Systematic Biology 41: 18–32.

Garland, T. Jr., Huey, R. B., & Bennett, A. F. 1991. Phylogeny and thermal physiology in lizards: a reanalysis. Evolution 45: 1969–1975.

Garraffo, H. M., Caeres, J., Daly, J. W., Spande, T. F., Andriamaharavo, N. R. & Andriantsiferana, M. 1993a. Alkaloids in Madagascan frogs (*Mantella*): pumiliotoxins, indolizidines, quinolizidines, and pyrrolizidines. Journal of Natural Products 56: 1016–1038.

Garraffo, H. M., Spande, T. F., Daly, J. W., Baldessari, A., & Gros, E. G. 1993b. Alkaloids from bufonid toads (*Melanophryniscus*): decahydroquinolines, pumiliotoxins, indolizidines, pyrrolizidines, and quinolizidines. Journal of Natural Products 56: 357–373.

Gould, S. J, & Lewontin, R. C. 1979. The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationists programme. Proceedings of the Royal Society of London B 205: 581–598.

Harvey, P. H., & Pagel, M. D. 1991. The comparative method and evolutionary biology. Oxford University Press, Oxford, England.

Ivlev, V.S. 1961. Experimental ecology of the feeding of fishes (Transl. by D. Scott). Yale University Press, New Haven.

Jacobs, J. 1974. Quantitative measurement of food selection: a modification of the forage ratio and Ivlev's electivity index. Oceologia 14: 413–417.

Jeffries, M. J. & Lawton, J. 1984. Enemy free space and the structure of ecological communities. Biological Journal of the Linnaean Society 23: 269–286.

Jones, T. H., Gorman, J. S. T., Snelling, R. R., Delabie, J. H. C., Blum, M. S., Garraffo, H. M., Jain, P., Daly, J. W., & Spande, T. F. 1999. Further alkaloids common to ants and frogs: decahydroquinolines and a quinolizidine. Journal of Chemical Ecology 25: 1179–1193.

La Marca, E. 1992. Catálogo taxonómico, biogeográfica y bibliográfico de las ranas de Venezuela. Mérida, Venezuela. Cuaderno Geográfico de Universidad de Los Andes 9: 1–197.

La Marca, E. 1994. Taxonomy of the frogs of the genus *Mannophryne* (Amphibia: Anura: Dendrobatidae). Publicaciones de la Asociación de Amigos de Doñana 4: 1-75.

Lüling, K. H. 1971. Der Fäberfrosch *Phyllobates bicolor* Bibron der Cordillera Azul (Peru). Bonner Zoologische Beitrage 22: 161–174.

Maddison, W. P. 1990. A method for testing the correlation of two binary characters: Are gains and losses concentrated on certain branches of a phylogenetic tree? Evolution 44: 539–557.

Martins, E. P. & Hansen, T. F. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. American Naturalist 149: 646–667.

Mebs, D. 2001. Toxicity in animals: trends in evolution? Toxicon 39: 87–96.

Myers, C. W. 1987. New generic names for some Neotropical poison frogs (Dendrobatidae). Papéis Avulsos de Zoologia de São Paulo 36: 301–306.

Myers, C. W. & Daly, J. W. 1976. Preliminary evaluation of skin toxins and vocalizations in taxonomic and evolutionary studies of poison-dart frogs (Dendrobatidae). Bulletin of the American Museum of Natural History 157: 174–262.

Myers, C. W., Paolillo O., A., & Daly, J. W. 1991. Discovery of a defensively malodorous and nocturnal frog in the family Dendrobatidae: phylogenetic significance of

a new genus and species from the Venezuelan Andes. American Museum Novitates 3002: 1–33.

Myers, C. W., Daly, J. W. & Malkin, B. 1978. A dangerously toxic new frog (*Phyllobates*) used by the Embera Indians of Western Colombia, with discussion of blowgun fabrication and dart poisoning. Bulletin of the American Museum of Natural History 161: 307–365.

Neter, J., Kutner, M. H., Nachtsheim, C. J., & Wasserman, W. 1996. Applied Linear Regression Models. Third Edition. R. D. Irwin, Illinois.

Neuwirth, M., Daly, J. W., Myers, C. W., & Tice, L.W. 1979. Morphology of the granular secretory glands in skin of poison-dart frogs (Dendrobatidae). Tissue & Cell 11: 755–771.

Nishida, R. 2002. Sequestration of defensive substances from plants by Lepidoptera. Annual Review of Entomology 47: 57–92.

Orians, G. H. & Paine, R. T. 1983. Convergent evolution at the community level, Pages 431–458, *in* D. J. Futuyma and M. Slatkin, eds., Coevolution. Sinauer, Sunderland, Massachusetts.

Pagel, M. 1993. Seeking the evolutionary regression coefficient: an analysis of what comparative methods measure. Journal of Theoretical Biology 164: 191–205.

Pagel, M. 1997. Inferring evolutionary processes from phylogenies. Zoologica Scripta 26: 331–348.

Pagel, M. 1999. Inferring the historical patterns of biological evolution. Nature 401: 877–884.

Parmelee, J. R. 1999. Trophic ecology of a tropical anuran assemblage. Scientific Papers of the Natural History Museum, University of Kansas 11: 1-59.

Pianka, E. R. 1986. Ecology and Natural History of Desert Lizards: Analyses of the Ecological Niche and Community Structure. Princeton University Press, Princeton, New Jersey.

Poulton, E. B. 1890. The Colours of Animals: Their Meaning and Use, Especially Considered in the Case of Insects. Kegan Paul, Trench, Trübner, London (International Scientific Series 68).

Purvis, A. & Rambaut, A. 1994. Comparative analysis by independent contrasts (CAIC). Version 2. Department of Zoology, Oxford University, Oxford.

Rowell-Rahier, M. 1984. The food plant preferences of *Phratora vitellinae* (Coleoptera: Chrysomelidae) A. Field observations. Oecologia 64: 369–374.

Santos, J. C., Coloma, L. A., & Cannatella, D. C. 2003 Multiple recurring origins of aposematism and diet specialization in poison frogs. Proceedings of the National Academy of Sciences USA 100: 12792–12797.

Saporito, R. A., Donnelly, M. A., Hoffman, R. L., Garraffo, H. M., & Daly, J. W. 2003. A siphonotid millipede (*Rhinotus*) as the source of spiropyrrolizidine oximes of dendrobatid frogs. Journal of Chemical Ecology 29: 2781–2786.

SAS Institute, Inc. 2000. JMP® Statistics and Graphics Guide, Version 4. SAS Institute Inc., Cary, North Carolina.

Schaffner, U., Boevé, J.-L., Gfeller, H., & Schlunegger, U. P. 1994. Sequestration of *Veratrum* alkaloids by specialist *Rhadinoceraea nodicornis* konow (Hymenoptera, Tenthredinidae) and its ecoethological implications. Journal of Chemical Ecology 20: 3233–3250.

Scudder, G. G. E., & Duffey, S. S. 1972. Cardiac glycosides in the Lygaeinae. Canadian Journal of Zoology 50: 35–52.

Sillén-Tullberg, B. 1993. The effect of biased inclusion of taxa on the correlation between discrete characters in phylogenetic trees. Evolution 47: 1182–1191.

Simon, M. P. & Toft, C. A. 1991. Diet specialization in small vertebrates: mite-eating in frogs. Oikos 67: 483–489.

Simpson, E. H. 1949. Measurement of diversity. Nature 163: 688.

Sokal, R. R. & Rohlf, F. J. 1981. Biometry: the principles and practice of statistics in biological research. 2nd Ed. W.H. Freeman and Co., San Francisco.

Spande, T. F., Garraffo, H. M., Edwards, M. W., Yeh, H. J. C., Pannell, L., & Daly, J. W. 1992. Epibatidine: a novel (chloropyridyl) azabicycloheptane with analgesic activity from an Ecuadorian poison frog. Journal of the American Chemistry Society 114: 3475–3478.

Spande, T. F., Jain, P., Garraffo, H. M., Pannell, L. K., Yeh, H. J. C., Daly, J. W., Fukumoto, S., Imamura, K., Tokuyama, T., Torres, J. A., Snelling, R. R., & Jones, T. H. 1999. Occurrence and significance of decahydroquinolines from dendrobatid poison frogs and a myrmicine ant: use of 1H and 13C NMR and their conformational analysis. Journal of Natural Products 62: 5–21.

Strong, D. R. 1979. Biogeographic dynamics of insect-host plant communities. Annual Review of Entomology 24: 89–120.

Summers, K. 2003. Convergent evolution of bright coloration and toxicity in frogs. Proceedings of the National Academy of Sciences USA 100: 12533–12534.

Summers, K. & Clough, M. E. 2001. The evolution of coloration and toxicity in the poison frog family (Dendrobatidae). Proceedings of the National Academy of Sciences USA 98: 6227–6232.

Szelistowski, W. A. 1985. Unpalatability of the poison arrow frog *Dendrobates pumilio* to the ctenid spider *Cupiennius coccineus*. Biotropica 17: 345–346.

Termonia, A., Pasteels, J. M., Windsor, D. M., & Milinkovitch, M. C. 2002. Dual chemical sequestration: a key mechanism in transitions among ecological specialization. Proceedings of the Royal Society of London B 269: 1–6.

Thompson, T. E. 1960. Defensive adaptations in opisthobranchs. Journal of the Marine Biological Association of the United Kingdom 39: 123–134.

Toft, C. A. 1980. Feeding ecology of thirteen syntopic species of anurans in a seasonal tropical environment. Oecologia 45: 131–141.

Toft, C. A. 1981. Feeding ecology of Panamanian litter anurans: patterns in diet and foraging mode. Journal of Herpetology 15: 139–144.

Toft, C. A. 1995. Evolution of diet specialization in poison-dart frogs (Dendrobatidae). Herpetologica 51: 202–216.

Vences, M., Glaw, F., & Böhme, W. 1997/98. Evolutionary correlates of microphagy in alkaloid-containing frogs (Amphibia: Anura). Zoologischer Anzeiger 236: 217–230.

Vences, M., Kosuch, J. , Boistel, R., Haddad, C. F., La Marca, E., Lötters, S., & Veith, M. 2003. Convergent evolution of aposematic coloration in Neotropical poison frogs: a molecular phylogenetic perspective. Organisms Diversity and Evolution 3: 215–226.

von Euw, J., Fishelson, J., Parsons, J. A. , & Reichstein, T. 1967. Cardenolides (heart poisons) in a grasshopper feeding on milkweeds. Nature 214: 3–39.

Chapter 3

A Mechanism for Diversity in Warning Signals: Conspicuousness Versus Toxicity in Poison Frogs*

Abstract: Many animals advertise their chemical defense to predators with conspicuous coloration and unpalatability, but little is known about the information in these signal elements. To effectively avoid predation, is it more advantageous to invest in increased conspicuousness, or greater noxiousness, or to allocate equally to both signal modalities? Using natural variation among poison frog species measured with spectral reflectance and toxicity assays, we tested the relative importance of warning signal components with predator-learning and avoidance experiments. Closely related species use alternative strategies: increasing either conspicuousness or toxicity affords equivalent avoidance by predators and provides protection to non-toxic mimic species. These equally effective predator avoidance tactics demonstrate different aposematic solutions for two potentially costly signal components, providing a mechanism for natural diversity in warning signals.

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3.1 INTRODUCTION

Escaping predation is essential to survival for most animals and has resulted in the evolution of an amazing diversity of predator avoidance tactics. Conspicuous coloration advertises anti-predator defense across many taxa, including invertebrates, fish, amphibians, snakes, and birds (Edmunds 1974; Ruxton et al. 2004). Such aposematic, or warning, signals are effective when predators associate color pattern with unprofitability and avoid the diagnostic coloration in subsequent encounters. Greater toxicity of brightly colored prey leads to faster avoidance learning by predators (Darst & Cummings 2006) and is thought to be proportional to the reduction in attack probability at each encounter (Turner et al. 1984). Similarly, predators learn faster to associate conspicuous, relative to cryptic, patterns with unpalatability (Gittleman & Harvey 1980; Roper & Redston 1987; Lindström et al. 1999). No study, however, has empirically evaluated the relative importance of these two components of aposematism, conspicuousness and unpalatability, for avoiding attack by predators. Do species avoid predation by investing in increased conspicuousness, or greater noxiousness, or do they allocate equally to both signal modalities? Here, we directly test the relative effectiveness of different combinations of warning signal components using natural variation among poison frog species.

Poison frogs (Dendrobatidae) display some of the most diverse warning signals in nature. Phylogenetic analyses indicate that an incredible variety of color combinations has arisen multiple times from cryptic ancestors in dendrobatid frogs (Santos et al. 2003;

Vences et al. 2003). To test the relative benefits of warning signal components, we exploited this natural variation in poison frogs from Ecuadorian Amazonia using three closely related, brightly colored, toxic species that differ in coloration, as well as species in a non-toxic clade of putative mimics (Bates 1862). We test the efficacy of this putative Batesian mimicry, as well as examine effects of the model's warning signal for protection afforded to each mimic.

We quantified interspecific variation among aposematic signal components, unpalatability and conspicuousness, using toxicity assays and spectral reflectance. In contrast to the expectation that the most conspicuous species will be the most noxious, we found the most toxic species is not the most conspicuous, whereas the most conspicuous species shows only moderate toxicity (Table 3.1). A diversity of skin alkaloids, which confer noxiousness, exists across poison frogs (Daly & Myers 1967; Daly et al. 1987; Daly 2003). We assessed species' relative toxicity using an assay of subcutaneous injection of frog skin extract into laboratory mice (Daly & Myers 1967; Darst & Cummings 2006). Conspicuousness is a function of a particular viewer's sensory system (Endler 1990), and, in aposematism, the most important viewer is the predator. Although accounts of predation on poison frogs are scarce, birds are potential predators (Master 1998; Summers 1999; Siddiqi et al. 2004). Accordingly, we evaluated conspicuousness of the three color patterns from a bird's eye view using an avian visual model that evaluates conspicuousness as a combination of color and brightness contrast (Vorobyev et al. 1998; Siddiqi et al. 2004; see Materials and Methods). In increasing

order of conspicuousness, the three color patterns examined are "yellow only," "red only," and "red + yellow." Each color pattern is found in a species of toxic *Epipedobates* and non-toxic *Allobates* (Figure 3.1). The unexpected pattern of variation that we uncovered in aposematic features allowed us to conduct controlled comparisons of the relative importance of conspicuousness and toxicity for warning signal effectiveness (i.e., one pair that differs significantly in conspicuousness but not in toxicity, and another pair that differs significantly in toxicity but not in conspicuousness).

We took advantage of this measured variation in conspicuousness and toxicity to examine the comparative saliency of warning signal components to predators and to test the effectiveness of mimetic convergence. We conducted predator learning and avoidance experiments using live frogs and naïve chicken predators. Predators were exposed to one of three learning stimuli in a series of learning trials: 1) high conspicuousness, moderate toxicity (*E. bilinguis*), 2) moderate conspicuousness, high toxicity (*E. parvulus*), or 3) moderate conspicuousness, moderate toxicity (*E. hahneli*). The degree to which predators avoid the aposematic individuals was assessed with pre-learning and post-learning choice trials. We then investigated whether convergence on the toxic *Epipedobates*' conspicuous coloration by non-toxic *Allobates* is effective for escaping predation: are these true Batesian mimics? This research experimentally tests, for the first time, the relative importance of the two components of aposematism for avoiding attack by predators, providing insight into different strategies of relative investment and yielding testable predictions for the evolution of warning signal diversity.

3.2 RESULTS

Variation in Unpalatability. Relative unpalatability of poison frog species was assessed using a toxicity assay because a quantitative assay for oral noxiousness does not exist. Species' relative toxicity was measured using a standard protocol of subcutaneous injection of frog skin extract into laboratory mice (Daly & Myers 1967; Darst & Cummings 2006). Time to recovery from injection of *E. parvulus* skin extract was significantly greater than that of either *E. bilinguis* or *E. hahneli* skin extract (Table 3.1; N = 5 mice per treatment; Kruskal-Wallis test; $Z_{parvulus\ -\ bilinguis,} = 2.507$, $p_{2\text{-tail}} = 0.012$; $Z_{parvulus\ –\ hahneli} = 2.507$, $p = 0.012$). The recovery times from injection of the less toxic species skin extracts were not significantly different from one another ($Z_{bilinguis\ -\ hahneli} = -1.571$, $p = 0.116$). Injection of *A. zaparo* Y, *A. zaparo* no Y, and *A. femoralis* skin extract caused no adverse reaction (no difference among reactions from *A. zaparo* and *A. femoralis* skin extracts and saline control injections; ANOVA, $p = 0.535$). These results demonstrate variation in chemical defense among *Epipedobates* species, and confirm the absence of alkaloids in *Allobates* (Daly 2003; Darst et al. 2005; Darst & Cummings 2006), suggesting an adaptive function for color pattern convergence (Figure 3.1).

Variation in Conspicuousness. Darst and Cummings (2006) demonstrated color pattern convergence by the two color morphs of *A. zaparo* (Y, no Y) to geographically localized models (*E. bilinguis* in the north and *E. parvulus* in the south). By converging on a toxic model's color pattern, the mimic is ultimately adopting the model's degree of visual

salience (conspicuousness). We evaluated conspicuousness of the three color patterns (red only, yellow only, and red + yellow) from a bird's eye view using an avian visual model that evaluated conspicuousness as a combination of color and brightness contrast (Vorobyev et al. 1998; Siddiqi et al. 2004). We calculated conspicuousness as the dorsal internal contrast comparing head, back, axilla and groin areas to side body accounting for the relative body area for each color patch (Figure 3.1). Hence, both color and brightness contrast (ΔS and ΔL, Figure 3.1) are weighted functions of the relative body area for each color patch, producing a measure of whole body conspicuousness that is more appropriate than single patch comparisons (Endler & Mielke 2005). Total conspicuousness was evaluated as vector distance in a perceptual space (i.e. Euclidean distance). We found that conspicuousness varies across species, and that each non-toxic *Allobates* has converged on the conspicuousness of a toxic, sympatric *Epipedobates* species (Figure 3.1, Table 3.1; Kruskal-Wallis test; $Z_{parvulus - zaparo \text{ no Y}} = -1.319$, $p = 0.187$; $Z_{bilinguis - zaparo \text{ Y}} = -0.184$, $p = 0.854$; $Z_{hahneli - femoralis} = 0$, $p = 1.00$). *Epipedobates bilinguis*, with both red and yellow color elements, is the most conspicuous of the toxic frogs, followed by *E. parvulus* and *E. hahneli*, each with single color elements, which do not differ significantly from one another in conspicuousness (Figure 3.1, Table 3.1; $Z_{bilinguis - parvulus} = -4.336$, $p < 0.001$; $Z_{bilinguis - hahneli} = -4.005$, $p < 0.001$; $Z_{parvulus - hahneli} = -1.606$, $p = 0.108$).

We found that the most toxic species, *E. parvulus* (red only), is not the most conspicuous, whereas the most conspicuous species, *E. bilinguis* (red + yellow), shows only moderate toxicity. *Epipedobates hahneli* (yellow only), displays moderate levels of

both signal components (Figure 3.1, Table 3.1). This unexpected pattern of variation allows for controlled comparisons of the relative importance of conspicuousness and toxicity for warning signal effectiveness (i.e., *E. bilinguis* and *E. hahneli*, which differ significantly in conspicuousness but not in toxicity; and *E. parvulus* and *E. hahneli*, which differ significantly in toxicity but not in conspicuousness). Interestingly, the color patterns of all three brightly colored, toxic species are mimicked by a non-toxic *Allobates*, suggesting Batesian mimicry (Figure 3.1, Table 3.1).

Effectiveness of Aposematic Signal Components for Avoiding Predation. We examined the relative contributions of conspicuous coloration and unpalatability to escaping predation with two measures: speed of avoidance learning and degree of avoidance after learning. Predator learning experiments were conducted using live frogs and naïve chicken predators in which predators were exposed to one of three learning stimuli in a series of learning trials: 1) high conspicuousness, moderate toxicity (*E. bilinguis*), 2) moderate conspicuousness, high toxicity (*E. parvulus*), or 3) moderate conspicuousness, moderate toxicity (*E. hahneli*). We found that speed of learning was mediated by toxicity. Predators learned most quickly on the most toxic frog (Figure 3.2; N = 6 chicks per treatment; *E. parvulus* mean learning slope, 40.33 ± 8.11; *E. bilinguis*, 18.04 ± 7.4; *E. hahneli*, 16.60 ± 2.36; $Z_{parvulus\ -\ bilinguis} = 1.992$, $p = 0.046$; $Z_{parvulus\ -\ hahneli} = 2.005$, $p = 0.045$). Toxic frogs were rejected with no harm to the predator suggesting that greater toxicity confers protection through increased unpalatability. Our results also showed that increased conspicuousness had no effect on learning speed: predators learned

at similar rates on highly and moderately conspicuous frogs of similar toxicity ($Z_{bilinguis - hahneli} = 0.7488$, $p = 0.810$).

Although the first measure, speed of avoidance learning, is important for protection from predation, the ultimate determination of advantage is the second measure, the degree to which predators avoid aposematic individuals (Ruxton et al. 2004). A classic and enduring argument for the advantage of conspicuousness is that bright coloration makes predators less likely to confuse toxic prey with palatable prey, which are typically cryptic (Fisher 1930; Sherratt & Beatty 2003). This argument is particularly applicable when predators do not show innate aversion to bright colored prey, which was the case with our naïve chick predators (pre-learning time spent by chicks in each frog's quadrant; *E. bilinguis* 34.17 ± 2.0, *Colostethus* 40.83 ± 4.9, $Z_{bilinguis- Colostethus} = 1.959$, $p = 0.375$; *E. parvulus* 49.17 ± 6.2, *Colostethus* 54.16 ± 8.9, $Z_{parvulus - Colostethus} = 1.959$, $p = 1.77$; *E. hahneli* 36.67 ± 7.5, *Colostethus* 34.17 ± 8.9, $Z_{hahneli- Colostethus} = 1.959$, $p = 0.582$). We tested the discriminability hypothesis with the pair of *Epipedobates* that vary significantly in conspicuous coloration but not in toxicity (*E. bilinguis* and *E. hahneli*; Table 3.1), allowing for the first direct, controlled test of the effectiveness of natural variation in conspicuousness. The degree to which predators avoid the aposematic individuals was assessed with post-learning choice trials. Having learned to associate conspicuous coloration with unpalatability, educated predators were given both a control frog, a cryptic, non-toxic dendrobatid (*Colostethus awa*), and the conspicuous, toxic frog with which the predator had been trained. As predicted (Fisher 1930; Sherratt & Beatty 2003), greater conspicuousness of *E. bilinguis* resulted in significantly greater avoidance

by educated predators (Figure 3.3; time spent with stimulus frog / time spent with control: *E. bilinguis* as stimulus, 0.12 ± 0.03; *E. hahneli*, 0.44 ± 0.34; $Z_{bilinguis - hahneli} = 2.732$, $p = 0.006$). High toxicity with moderate conspicuousness proved to be an equally effective combination. *Epipedobates parvulus*, the most toxic species, garnered the same degree of avoidance as the more conspicuous *E. bilinguis* (Figure 3.3; E. *parvulus*, 0.13 ± 0.03; $Z_{parvulus - bilinguis} = -0.161$, $p = 0.872$; $Z_{parvulus - hahneli} = -2.566$, $p = 0.010$). Hence, high toxicity with moderate conspicuousness and moderate toxicity with high conspicuousness are equally successful signal component combinations for achieving effective predator avoidance.

Batesian Mimicry. Having demonstrated convergence on toxic frogs' conspicuous coloration by non-toxic *Allobates*, we tested whether this mimicry is effective for escaping predation: is convergence on conspicuousness functional Batesian mimicry? We found that the mimics successfully deceive predators. Chick predators trained with each model avoided the respective mimic as well, an empirical confirmation of Batesian mimicry by not one, but two closely related species in a distantly related clade. Mimics of either the more toxic or more conspicuous model received the high degree of avoidance afforded to their respective model (Figure 3.3; *A. zaparo* no Y, 0.08 ± 0.01, $Z_{parvulus - zaparo \text{ no } Y} = 1.046$, $p = 0.295$; *A. zaparo* Y as stimulus, 0.15 ± 0.03, $Z_{bilinguis - zaparo\ Y} = -0.646$, $p = 0.518$). Accordingly, the mimic of the moderately conspicuous and moderately toxic frog received the same moderate degree of avoidance afforded to its model, significantly less than that conferred to *A. zaparo* Y and no Y (Figure 3.3; *A.*

femoralis, 0.46 ± 0.06; $Z_{femoralis - zaparo\ Y} = -2.802$, $p < 0.005$; $Z_{femoralis - zaparo\ no\ Y} = -2.807$, $p < 0.005$).

3.3 DISCUSSION

Our results uncover different aposematic solutions to effectively avoid predation that take advantage of the relative benefits of toxicity and conspicuousness. Predators learn more quickly to avoid highly versus moderately toxic prey; whereas, an increase to greater conspicuousness does not increase the speed of learning. However, enhancing the complexity of the prey environment with both conspicuous and cryptic prey, the advantage of increased conspicuousness becomes apparent. The benefit of increasing conspicuousness, independent of toxicity, is a significant gain in protection from predation, suggesting that conspicuous coloration helps predators distinguish toxic from palatable prey. We find that poison frog species use different combinations to achieve the same effect; equal protection is achieved with a combination of moderate toxicity and high conspicuousness as with high toxicity and moderate conspicuousness. Our findings reveal equally effective aposematic strategies, providing a mechanism for natural diversity in warning signals (Figure 3.4).

Aposematism succeeds when predators associated conspicuousness with unprofitability, and in dendrobatid frogs, multiple origins of conspicuousness are correlated with multiple acquisitions of toxicity (Santos et al. 2003). During origins of

aposematism (evolutionary transitions from cryptic to aposematic), a positive correlation between conspicuousness and the strength of defense is predicted (Ruxton et al. 2004), and has been reported (Summers & Clough 2001). Our empirical data suggest that after this correlation is achieved, degree of conspicuousness and level of defense may become dissociated and adjusted independently. We find that conspicuousness and unpalatability are decoupled: *E. bilinguis* and *E. hahneli* differ significantly in conspicuousness but not in toxicity, whereas *E. parvulus* and *E. hahneli* differ significantly in toxicity but not in conspicuousness (Figure 3.4). Our results suggest the hypothesis of a trade-off between the two components of aposematism for effectively and efficiently escaping predation. Theoretical work has anticipated cross-compensation between potentially costly unprofitability and bright coloration, predicting that optimal investment in secondary-defense will diminish when more cost-effective conspicuousness evolves as primary defense (Leimar et al. 1986; Speed & Ruxton 2005a). There will, however, be constraints in how signal components can be adjusted, particularly in cases of Müllerian mimicry and limited genetic variability. Theoretical predictions and our results support a dynamic, complex relationship between signal components that should be further investigated.

The relative costs of increased conspicuousness versus high toxicity remain unknown, although growing empirical evidence indicates that chemical defenses are costly in a variety of circumstances (Ruxton et al. 2004). Additionally, complete dissociation of conspicuousness and toxicity in Batesian mimics suggests that if warning

coloration can be exploited without investment in noxiousness, then Batesian mimicry is the preferred strategy. The noxious alkaloids in the skin of poison frogs are sequestered from a specialized diet of leaf litter arthropods (Daly 2003 and references therein; Darst et al. 2005). An animal that accumulates toxic metabolites not only has to ingest toxic prey (Duffey 1980) but also is restricted to a specialized diet (Mebs 2001). If the cost of either diet specialization or sequestration becomes too great (for example, with change in prey resources), shedding the expense of high toxicity in favor of increased conspicuousness may be a more efficient predator avoidance tactic. This depends, however, on the relative costs of conspicuousness due to acquiring or producing conspicuous pigmentation or simply the cost of raised apparency to predators, which is unknown in poison frogs. Moderate levels of toxicity and conspicuousness may be favored when costs associated with high levels of signal components are disadvantageous and moderate protection is sufficient. Such a selective advantage may occur when a surplus of palatable, non-toxic prey is available and predators, therefore, only rarely resort to moderately toxic prey (Sherratt et al. 2004). Thus, the fitness benefits of moderate toxicity and moderate conspicuousness may be dependent upon the availability of alternative, non-toxic prey, which generates predictions that are testable in the field.

Warning coloration would initially be favored only after the acquisition of chemical defense, suggesting that conspicuous mutants arise from defended cryptic species (Poulton 1890; Cott 1940). New aposematic forms, therefore, will be selected against because of their conspicuousness and rarity (Speed & Ruxton 2005b). Interestingly, in poison frogs, the benefits of signaling may be conferred by individual

selection; we found that 79.24% ± 1.78 of frogs sampled by predators survived the attack, i.e. were tasted by the chick and promptly rejected with no harm to the frog (N = 62 sampled frogs). Hence, individuals with novel combinations of the two signal components are able to survive and reproduce, providing greater evolutionary lability in aposematic signals.

This research is the first to empirically tease apart the relative importance of the two components of aposematism for avoiding attack by predators. Our results demonstrate alternative strategies for combining toxicity and conspicuousness, suggesting that decoupling warning signal components enables effective and efficient predator avoidance and provides a mechanism for the generation and maintenance of diversity in aposematism. We hypothesize a trade-off between conspicuous coloration and unpalatability in achieving protection from predation and suggest a role for other ecological factors such as availability of alternative prey. Further information on the relative costs of signal components will improve our understanding of forces that generate variation in aposematism. Our results provide insight into different aposematic solutions of relative investment and yield testable predictions for the evolution of warning signal diversity.

3.4 MATERIALS AND METHODS

Collection. Dendrobatid frogs were collected in the Amazonian lowland rainforest and Western Andean slopes of Ecuador, January–May 2003, 2004, and 2005. The five collection sites were Estación Científica Yasuní, Francisco de Orellana Province (*Epipedobates hahneli* and *Allobates femoralis*); Estación Biológica Jatun Sacha, Napo Province (*E. bilinguis* and *A. zaparo* Y); Río Santiago, ~1 km E of Santiago, Morona-Santiago Province (*E. parvulus* and *A. zaparo* no Y); Río Toachi, ~2 km N of La Unión del Toachi, Pichincha Province (*Colostethus awa*). Taxonomy follows Frost (2004).

Unpalatability. Five frogs from each *A. femoralis*, *A. zaparo* Y, *A. zaparo* no Y, *E. bilinguis*, *E. hahneli*, and *E. parvulus* were euthanized and skinned following (Darst et al. 2005). All specimens were deposited at Museo de Zoología, Pontificia Universidad Católica del Ecuador (QCAZ). Toxicity assay methods follow (Darst & Cummings 2006). Methanol extracts from individual frog skins were evaporated to dryness and re-dissolved in sterile saline (~1ml saline / skin extract). Resultant alkaloid fractions were subcutaneously injected into seven treatments of five mice each (Daly & Myers 1967; Darst & Cummings 2006): single-skin extracts of (1) *A. femoralis*; (2) *A. zaparo* Y; (3) *A. zaparo* no Y; (4) *E. bilinguis*; (5) *E. hahneli*; (6) *E. parvulus*; or (7) saline-control injection. Each mouse was injected with extract of one frog skin or saline control (N = 35 mice, IACUC #03110501). Sleeping behavior was used as a baseline for all toxicity assays. Mice were awakened with injection and time to complete recovery (return to sleep) was recorded. Mouse recovery time following injection was used to estimate

degree of toxicity (Table 3.1). Skin extracts were only marginally "toxic" given that only one mouse died in our assay (death was from injection of an *E. parvulus* skin extract; because this mouse did not yield a time to recovery, its data were removed from the analysis). Thus, we use the term "toxicity" to refer to relative irritant effect of frog skin alkaloids and as a proxy for unpalatability. We used a Kruskal-Wallis non-parametric test for all comparisons between recovery times because variances did not meet homogeneity assumptions of parametric tests. ANOVA was used to compare recovery times among groups.

Conspicuousness. Eighty poison frogs were collected and transported to Museo de Zoología, Pontificia Universidad Católica del Ecuador (QCAZ) for reflectance measurements (*E. parvulus*: N = 16, *A. zaparo* no Y: N = 12; *E. bilinguis*: N = 16; *A. zaparo* Y: N = 15; *E. hahneli*: N = 10, *A. femoralis*: N = 11). Spectral reflectances were measured using an Ocean Optics PS2000 spectrometer, full spectrum light source (DT-1000), Spectralon white standard, and reflectance probe (R400-7) at 2 mm distance from eight body-regions: head, dorsum, left and right axillas, groins, and flanks (side body) with two measurements per region. Twenty samples of leaf-litter found near or upon where frogs were first sighted were collected. Spectral reflectances of frogs' leaf litter background was measured using the same protocol as above. Available light in the forest (habitat spectral irradiance) measurements were collected at 0900 hrs on 9 different days with the PS2000 and cosine collector connected to a 400 μm fiber optic. Frog and background radiance estimates were computed as the product of spectral reflectances and the average habitat irradiance spectrum for all locations. Frog dorsal coloration was

computed as a combination of head, dorsum, axilla, and groin radiances weighted by body area.

To evaluate the conspicuousness of the different color patterns, we used an avian tetrachromatic visual model following (Vorobyev et al. 1998) that includes both a chromatic (color) and achromatic (brightness) channel as in (Siddiqi et al. 2004). We evaluated conspicuousness in terms of spectral (ΔS) and brightness (ΔL) contrast for internal contrast. The avian vision model was used to describe color and brightness discrimination where vision is limited by photoreceptor noise. The model begins with photoreceptor photon capture (cone quantum catch), Q_c, which represents a certain level of excitation for cone class, c, while viewing target, t, stimuli under specific irradiance measurements: $Q_c = \sum_{\lambda=300}^{700} I_i(\lambda)R_t(\lambda)A_c(\lambda)$. Cone quantum catch of target radiances, Q_c, is evaluated as the summed product of illuminating irradiance $I_i(\lambda)$; target reflectance, $R_t(\lambda)$; and the absorptance spectrum (including ocular or screening pigments where appropriate), $A_c(\lambda)$, for a given photoreceptor cone class c. These photon capture responses are then adjusted for the adapting background light through a process known as the von Kries transformation, where $q_c = k_c Q_c$, and $k_c = 1/\sum_{\lambda=300}^{700} I_b(\lambda)A_c(\lambda)$ where $I_b(\lambda)$ is the irradiance of the adapting visual background.

The next stage in this visual model assumes that photoreceptors adaptation follows the weber-fechner laws (Vorobyev et al. 1998; Chiao et al. 2000), where the signal of each cone channel is proportional to the logarithm of the background adjusted

quantum catch: $f_c = \ln(q_c)$. Color differences between frog body color reflectances were evaluated as the receptor (cone class) channel differences normalized by noise in each receptor channel (e.g. $\Delta f_c = \ln(q_L[\text{red back}]) - \ln(q_L[\text{side body}])$). Noise in each receptor channel, ω_c , is assumed to be independent of quantal fluctuations and was set by the relative number of receptor types within a typical avian receptive field ($\omega_U = 1.0$; $\omega_S = 0.857$; $\omega_M = 0.520$; $\omega_L = 0.515$; where U = Ultraviolet sensitive ; S = Shortwave sensitive; cone proportions from Hart et al. 1998).

The spectral distance, ΔS, or the distance separating two spectra in perceptual space is defined as:

$$
\begin{aligned}
(\Delta S)^2 = {} & (\omega_U\omega_S)^2(\Delta f_L - \Delta f_M)^2 + (\omega_U\omega_M)^2(\Delta f_L - \Delta f_S)^2 + \\
& (\omega_U\omega_L)^2(\Delta f_M - \Delta f_S)^2 + (\omega_S\omega_M)^2(\Delta f_L - \Delta f_U)^2 + \\
& (\omega_S\omega_L)^2(\Delta f_M - \Delta f_U)^2 + (\omega_M\omega_L)^2(\Delta f_S - \Delta f_U)^2 / \\
& \left((\omega_U\omega_S\omega_M)^2 + (\omega_U\omega_S\omega_L)^2 + (\omega_U\omega_M\omega_L)^2 + (\omega_S\omega_M\omega_L)^2\right)
\end{aligned}
$$

Brightness contrast, or the achromatic processing channel, of the avian visual system is considered to be a function of the double cone class that represents the absorption spectra of long-wavelength sensitivity (LWS) cone photoreceptors (Siddiqi et al. 2004). Brightness for the potential bird predators in this system was therefore for LWS cones ($L = f_L$), and brightness contrast estimates, ΔL, were evaluated as the absolute difference between two color elements: $\Delta L = |(L_1 - L_2)/\omega_L|$.

We evaluated conspicuousness in terms of spectral (ΔS) and brightness (ΔL) contrast for internal contrast viewed dorsally (as by a potential avian predator) by comparing head, back, axilla and groin areas to flanks (side body) accounting for the relative body area for each color patch. We used photographs of model frogs viewed from above to estimate the percent body area of each color patch in Adobe Photoshop with head and dorsal regions accounting for 88% and remaining areas 12%. Hence, each ΔS and ΔL is a weighted function of the relative body area for each color patch, producing a measure of whole body conspicuousness that is more appropriate than single patch comparisons (Endler & Mielke 2005). Conspicuousness viewed from above was evaluated as the Euclidean distance of color and brightness contrast, $E = \sqrt{\Delta S^2 + \Delta L^2}$, producing vector distance in a perceptual space (Table 3.1). Confidence ellipses (95%) were calculated for each species (Figure 3.1; *E. parvulus*: N = 24, *A. zaparo* no Y: N = 11; *E. bilinguis*: N = 19; *A. zaparo* Y: N = 17; *E. hahneli*: N = 10, *A. femoralis*: N = 12). We used a Kruskal-Wallis non-parametric test for all comparisons between Euclidean distances.

Predator learning experiments. Predator learning experiments generally followed methods as described in Darst & Cummings (2006). While few data exist, birds may be potential poison frog predators (Master 1998; Summers 1999; Siddiqi et al. 2004). Thus, in Quito, Ecuador, we conducted a series of learning experiments using ~1 month old domestic chickens (*Gallus gallus domesticus*) as naïve, model predators (Osorio et al. 1999) and wild-caught dendrobatids (toxic species: *E. bilinguis*, *E. hahneli* and *E.*

parvulus; non-toxic species: *A. femoralis*, *A. zaparo* Y, and *A. zaparo* no Y). Birds were tested individually in a 1 m^2 dirt-floor test-arena of four 50 cm^2 quadrants outside, under natural lighting conditions. Chickens were fed chicken mash and cracked corn twice daily, and water *ad libitum*. We assessed *Allobates* palatability by presenting nine naïve chickens an *Allobates* (three *A. femoralis*, three *A. zaparo* Y, and three *A. zaparo* no Y). Naïve chickens readily ate all *Allobates* and control frogs (*C. awa*). We assessed the effects of conspicuousness on innate predator behavior (baseline) with pre-learning choice experiments in which the brightly colored learning stimulus species was paired with a cryptic control frog. Chicks were presented with both the brightly colored frog and control frog, each under a glass dome, for two minutes; time spent in each dome's test-arena quadrant was recorded.

We had three experimental groups (6 chicks each), differing in learning stimulus species (*E. bilinguis*, *E. hahneli* or *E. parvulus*), in 8 learning trials (IACUC # 04071901). A learning trial consisted of presenting a chick with a learning-stimulus under a glass dome for 1 min or until the chick pecked the dome. The dome was then removed and latency to peck the stimulus was recorded up to 2 minutes or until first peck (sampling event) (Figure 3.2). A typical sampling event involved the chick grabbing the frog in its beak and spitting the frog out. Only one chick fully ingested a poison frog (*E. bilinguis*). This animal died three days later and its data were therefore removed from the experiment. We defined learning rate as the slope (latency to peck / # of trials) until full learning (no subsequent sampling in further trials). Learning slopes were compared using

a Kruskal-Wallis test. Control frogs were presented to chicks after trials #2 and #6 to ensure chicks were still motivated to eat frogs.

After training was complete, degree of avoidance was assessed in two choice experiments, one that paired the control frog with the toxic learning-stimulus model (the same choice as in the pre-learning trial) and the second that paired the control with the appropriate *Allobates* mimic of the learning-stimulus. Chicks were presented with both the brightly colored frog and control frog, each under a glass dome for two minutes; time spent in each dome's test-arena quadrant was recorded. Placement of frogs within the test arena was randomized across trials. We assessed learned avoidance by comparing time spent by the predator with the learning stimulus to the time spent with the control frog in post-learning choice trials (Figure 3.3). Protection from predation was measured as the ratio of pre-learning (baseline) to post-learning time spent with the stimulus frog (Figure 3.4). All comparisons were made using a Kruskal-Wallis test.

Table 3.1. Natural variation in toxicity and conspicuousness among model-mimic pairs (*Epipedobates*-*Allobates*).

Learning stimulus	Toxicity (mins)[a]	Relative toxicity[b]	Conspicuousness (*E*)[c]	Relative conspicuousness[b]
E. bilinguis	79.00 ± 3.189	0.60 ± 0.039	2.46 ± 0.180	1.00 ± 0.077
A. zaparo Y	4.80 ± 0.183	0.04 ± 0.002	2.43 ± 0.260	0.97 ± 0.104
E. parvulus	135.40 ± 9.312	1.00 ± 0.070	1.45 ± 0.067	0.58 ± 0.027
A. zaparo no Y	5.25 ± 0.391	0.03 ± 0.003	1.30 ± 0.114	0.52 ± 0.045
E. hahneli	68.20 ± 2.935	0.51 ± 0.022	1.16 ± 0.139	0.46 ± 0.056
A. femoralis	4.80 ± 0.342	0.04 ± 0.002	1.16 ± 0.111	0.46 ± 0.044

All data are mean ± SE.

[a] Toxicity is measured in time (minutes) to recovery from subcutaneous injection of frog skin extract into laboratory mice.

[b] Relative toxicity and conspicuousness are scaled to a maximum of 1.00.

[c] Conspicuousness is measured as the Euclidean distance ($E = \sqrt{\Delta S^2 + \Delta L^2}$) of color (ΔS) and brightness (ΔL) contrast of weighted dorsal coloration to side coloration (internal contrast).

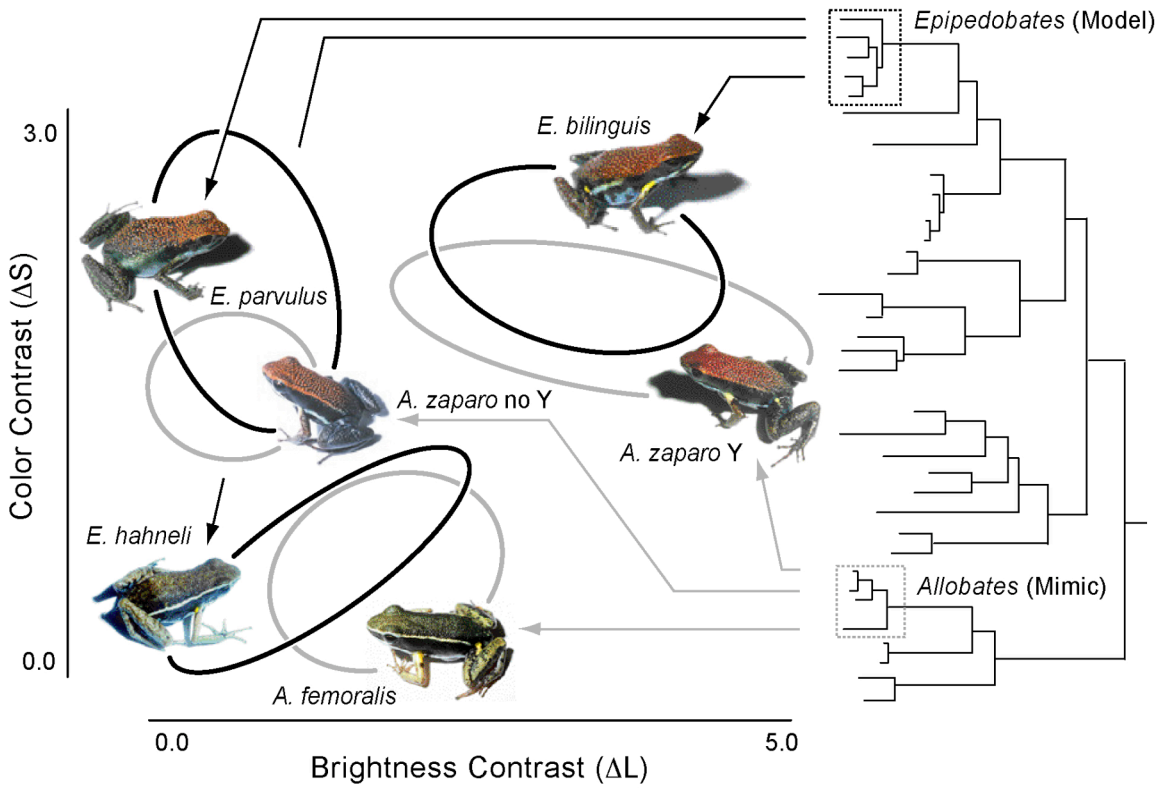

Figure 3.1. Conspicuousness of poison frog species as viewed by a potential avian predator. *Epipedobates bilinguis* (N = 16) and sympatric *Allobates zaparo* Y (N = 15) have a mostly red, granular dorsum with yellow blotches in axilla and groin regions (red + yellow); *E. parvulus* (N = 16) and *A. zaparo* no Y (N = 12) have a red dorsum, but lack the yellow regions (red only); and *E. hahneli* (N = 10) and *A. femoralis* (N = 11) have a dark brownish dorsum with the yellow blotches in the axilla and groin (yellow only). The *y*-axis is color contrast (ΔS = spectral discrimination) and the *x*-axis is brightness contrast (ΔL = long wavelength sensitivity cone contrast) as computed using frog color radiances in an avian visual model (Vorobyev et al. 1998; Siddiqi et al. 2004).

Conspicuousness is based on dorsal internal contrast comparing head, back, axilla and groin areas to side body accounting for the relative body area for each color patch. Ellipses show 95% confidence intervals for each species; the ellipse of each mimic (gray) overlaps with each respective model species (black). Phylogeny of Dendrobatidae is adapted from Santos et al. (2003).

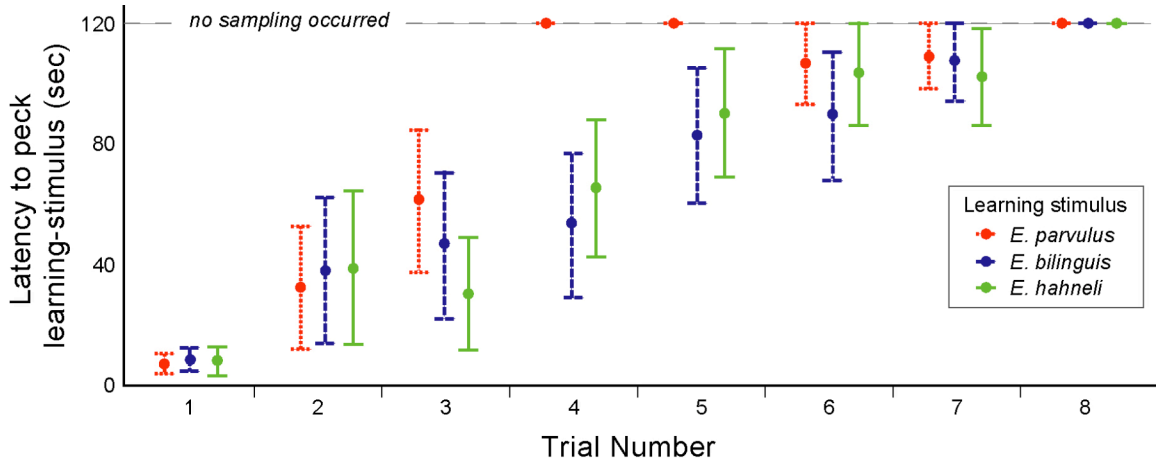

Figure 3.2. Predators learn not to attack toxic, conspicuous poison frogs over a series of learning trials. Learning proceeded fastest with the most toxic frog (*E. parvulus* evoked full learning by trial 4.33 ± 0.95 (SE); *E. bilinguis,* 6.33 ± 0.99; *E. hahneli*, 6.55 ± 0.56). A learning trial (*x*-axis) consisted of presenting chicks with one of the brightly colored, toxic frogs under a glass dome for 1 minute or until chicks pecked the dome; the dome was then removed and latency to peck the stimulus (sampling event) was recorded up until 120 seconds (*y*-axis). Data are mean ± standard deviation (N = 6 chicks per treatment).

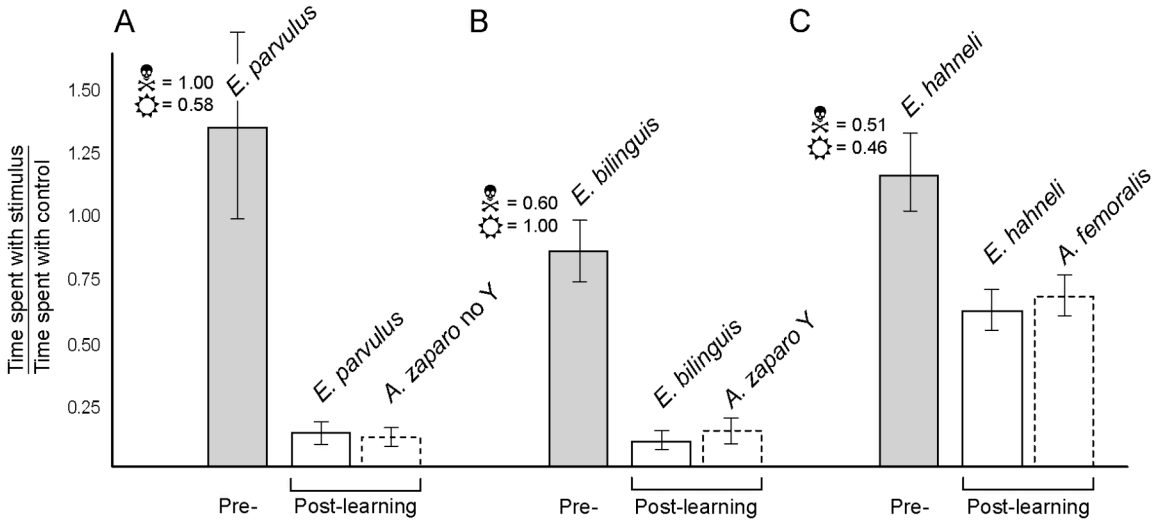

Figure 3.3. Educated predators avoid the toxic, conspicuous *Epipedobates* species and their respective *Allobates* mimics. The *y*-axis represents the relative time spent by predators with the brightly colored frog (stimulus) in pre- and post-learning choice trials (*x*-axis) (data are mean ± SE; N = 6 chicks per treatment); significance was measured comparing pre- to post-learning avoidance (in all cases: $Z = 2.802$, $p_{2\text{-tail}} < .005$). Skull and crossbones icons represent relative toxicity; sun icons represent relative conspicuousness (Table 3.1). **(A, B, C)** Chicks spent significantly less post-learning than pre-learning time with toxic frogs, which is conferred to each respective non-toxic mimic (bars outlined by dashes). **(C)** The degree of avoidance received by *E. hahneli* and *A. femoralis* was significantly less than the degree of avoidance provided to more conspicuous *E. bilinguis* or more toxic *E. parvulus* and their mimics ($Z_{hahneli\ -\ parvulus} = 2.566$, $p_{2\text{-tail}} = 0.010$; $Z_{hahneli\ -\ bilinguis} = 2.7312$, $p_{2\text{-tail}} = 0.006$).

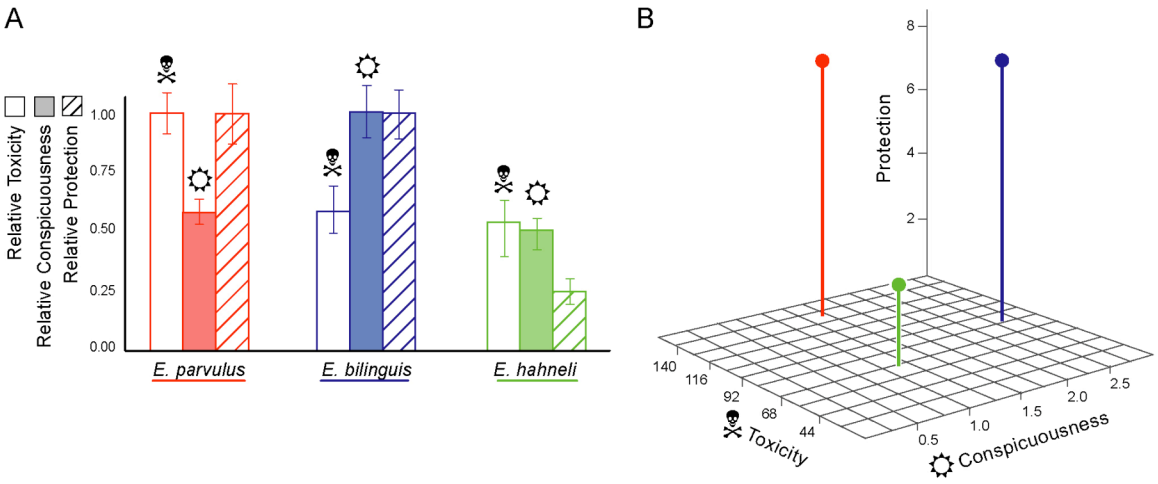

Figure 3.4. Conspicuous, toxic poison frogs achieve equal protection from predation with different combinations of warning signal components. Skull and crossbones icons represent toxicity; sun icons represent conspicuousness. Protection from predation is measured as the ratio of pre-learning time to post-learning time spent with the stimulus frog. (A) *Epipedobates parvulus* achieves equal protection from predation with high toxicity and moderate conspicuousness as *E. bilinguis* achieves with moderate toxicity and high conspicuousness. Relative toxicity, conspicuousness, and protection are scaled to a maximum of 1.00 (Table 3.1; data are mean ± SE; N = 6 chicks per treatment). (B) The comparative benefits of warning signal components, conspicuousness and toxicity, support alternative strategies for an effective and efficient warning signal. Measured (non-relative) data for toxicity, conspicuousness, and protection from predation are shown.

References

Bates, H. W. 1862. Contributions to an insect fauna of the Amazon valley. Lepidoptera: Heliconidae. Transactions of the Linnean Society of London 23: 495–566.

Chiao, C.-C., Vorobyev, M., Cronin, T., Osorio, D. 2000. Spectral tuning of dichromats to natural scenes. Vision Research 40: 3257–3271.

Cott, H. B. 1940. Adaptive coloration in animals. London: Methuen.

Daly, J. W. 2003. Amphibian skin: a remarkable source of biologically active arthropod alkaloids. Journal of Medical Chemistry 46: 445–452.

Daly, J. W. & Myers, C. W. 1967. Toxicity of Panamanian poison frogs (*Dendrobates*): some biological and chemical aspects. Science 156: 970–973.

Daly, J. W., Myers, C. W., & Whittaker, N. 1987. Further classification of skin alkaloids from Neotropical poison frogs (Dendrobatidae), with a general survey of toxic noxious substances in the Amphibia. Toxicon 25: 1023–1095.

Darst, C. R., & Cummings, M. E. 2006. Predator learning favours mimicry of a less toxic model in poison frogs. Nature. 404: 208–211.

Darst, C. R., Menéndez-Guerrero, P. A., Coloma, L. A., & Cannatella, D. C. 2005. Evolution of dietary specialization and chemical defense in poison frogs (Dendrobatidae): a comparative analysis. American Naturalist. 165: 56–69.

Duffey, S. S. 1980. Sequestration of plant natural products by insects. Annual Review of Entomology 25: 447–477.

Edmunds, M. 1974. *Defence in Animals: A Survey of Anti-predator defences*. New York: Longman Group Limited.

Endler, J. A. 1990. On the measurement and classification of colour in studies of animal colour patterns. Biological Journal of the Linnean Society 41: 315–352.

Endler, J. A. & Mielke, P. W. 2005. Comparing entire colour patterns as birds see them. Biological Journal of the Linnean Society 86: 405–431.

Fisher, R. A. 1930. *Genetical theory of natural selection* Oxford: Clarendon Press.

Frost, D. R. 2004. Amphibian species of the world: an online reference, V 3.0. http://research.amnh.org/herpetology/amphibia/index.html; American Museum of Natural History, NY.

Gittleman, J. L. & Harvey, P. H. 1980. Why are distasteful prey not cryptic? Nature 286: 149–150.

Hart, N. S., Partridge, J. C., Cuthill, I. C. 1998. Visual pigments, oil droplets and cone photoreceptor distribution in the European starling (*Sturnus vulgaris*) Journal Experimental Biology 201: 1433–1446.

Leimar, O., Enquist, M., Sillén-Tullberg, B. 1986. Evolutionary stability of aposematic coloration and prey unprofitability: a theoretical analysis. American Naturalist 128: 469–490.

Lindström, L., Alatalo, R. V., Mappes, J., Riipi, M., Vertainen, L. 1999. Can aposematic signals evolve by gradual change? Nature 397: 249–251.

Master, T. L. 1998. *Dendrobates auratus* (black and green poison dart frog): Predation. Herpetological Review 29: 164–165.

Mebs, D. 2001. Toxicity in animals: trends in evolution? Toxicon 39: 87–96.

Osorio, D., Vorobyev, M. & Jones, C. D. 1999. Colour vision of domestic chicks. Journal of Experimental Biology 202: 2951–2959.

Poulton, E. B. 1890. The Colours of Animals: Their Meaning and Use, Especially Considered in the Case of Insects. Kegan Paul, Trench, Trübner, London (International Scientific Series 68).

Roper, T. J., & Redston, S. 1987. Conspicuousness of distasteful prey affects the strength and durability of one-trial avoidance learning. Animal Behaviour 35: 739–747.

Ruxton, G. D., Sherratt, T. N., & Speed, M. P. 2004. *Avoiding attack: the evolutionary ecology of crypsis, aposematism, and mimicry*. Oxford: Oxford University Press.

Santos J. C., Coloma L. A., & Cannatella D. C. 2003. Multiple recurring origins of aposematism and diet specialization in poison frogs. Proceedings of the National Academy of Sciences USA 100: 12792–12797.

Sherratt, T. N. & Beatty, C. D. 2003. The evolution of warning signals as reliable indicators of prey defense. American Naturalist 162: 377–389.

Sherratt, T. N., Speed, M. P., & Ruxton, G. D. 2004. Natural selection on unpalatable species imposed by state-dependent foraging behaviour. Journal of Theoretical Biology 228: 217–226.

Siddiqi, A., Cronin, T. W., Loew, E. R., Vorobyev, M., & Summers, K. 2004. Interspecific and intraspecific views of color signals in the strawberry poison frog *Dendrobates pumilio*. Journal of Experimental Biology 207: 2471–2485.

Speed, M. P, & Ruxton, G. D. 2005a. Warning displays in spiny animals: one (more) evolutionary route to aposematism. Evolution 59: 2499–2508.

Speed, M. P, & Ruxton, G. D. 2005b. Aposematism: what should our starting point be? Proceedings of the Royal Society of London B: 272: 431–438.

Summers, K. 1999. *Dendrobates auratus* (green poison frog): Predation. Herpetological Review 30: 91.

Summers, K. & Clough, M. E. 2001. The evolution of coloration and toxicity in the poison frog family (Dendrobatidae). Proceedings of the National Academy of Sciences USA 98: 6227–6232.

Turner, J. R. G., Kearney, E. P., & Exton, L. S. 1984. Mimicry and the Monte Carlo predator: the palatability spectrum and the origins of mimicry. Biological Journal of the Linnean Society 23: 247–268.

Vences, M., Kosuch, J., Boistel, R., Haddad, C. F., La Marca, E., Lötters, S., & Veith, M. 2003. Convergent evolution of aposematic coloration in Neotropical poison frogs: a molecular phylogenetic perspective. Organismal Diversity and Evolution 3: 215–226.

Vorobyev, M., Osorio, D., Bennett, A. T. D., Marshall, N. J., & Cuthill, C. 1998. Tetrachromy, oil droplets and bird plumage colors. Journal of Comparative Physiology A 183: 621–633.

Chapter 4

Predator learning favours mimicry of a less toxic model in poison frogs*

Abstract. Batesian mimicry, resemblance of a toxic model by an edible mimic, depends on deceiving predators (Bates 1862). Mimetic advantage is considered to be frequency-dependent because increased mimic abundance leads to warning signal breakdown (Fisher 1930; Brower & Brower 1962). Where multiple toxic species are available, Batesian polymorphism (Wallace 1865) is predicted—mimics diversify to match sympatric models. Despite the prevalence of Batesian mimicry in nature (Edmunds 1974), Batesian polymorphism is relatively rare (for review: Joron & Mallet 1998). Here we explore a poison frog mimicry complex composed of two parapatric models and a geographically dimorphic mimic that exhibits monomorphism where models co-occur. Contrary to classical predictions, our toxicity assays, field observations, and spectral reflectances show mimics resemble the less toxic and less abundant model. We examine "stimulus generalization" (Pavlov 1927) as a mechanism for this non-intuitive result with learning experiments using naïve avian predators and live poison frogs. Predators differed in avoidance generalization depending on model toxicity, conferring greater protection to mimics resembling the less toxic model due to overlap of generalized avoidance curves. Our work supports a mechanism of toxicity-dependent stimulus generalization (Duncan & Sheppard 1965), revealing an additional solution for Batesian mimicry where multiple models coexist.

*Significant portions of this chapter have been previously published as Darst & Cummings, 2006. Nature 404: 208–211.

4.1 BATESIAN MMICRY OF A LESS TOXIC MODEL

In Batesian mimicry, an edible species co-opts an unpalatable species' warning signal to gain advantage through predator deception (Bates 1862). If Batesian mimics are too common, however, this advantage breaks down as predators learn to ignore the warning signal. Where more than one model species is available (Wallace 1865), diversifying frequency-dependent selection predicts the evolution of polymorphism in which mimics diverge in appearance to resemble sympatric models (Ford 1971; Tuner 1987; Joron and Mallet 1998). Batesian polymorphism is suggested to distribute warning signal degradation over several defended model species, allowing the mimic to increase in abundance. Reported accounts of such mimetic polymorphism, however, are relatively rare[6] and unknown in vertebrate mimicry systems (Brodie & Brodie 1980; Greene & McDiarmid 1981). Here we investigate a mimicry system that is inconsistent with the predictions of frequency-dependence. We examine a poison frog mimicry complex composed of two parapatric models and a geographically varying mimic (Figure 4.1).

The model Ecuadorian poison frogs *Epipedobates bilinguis* and *E. parvulus* share a similar warning signal of a bright red-spotted dorsum but differ in axilla and groin colouration (Figure 4.1b). Their phylogenetically distant relative (Santos et al. 2003),

Allobates zaparo, is geographically dimorphic, matching each warning signal where models are parapatric (Figure 4.1b). Where the two models co-occur, however, the mimic resembles only a single model (*E. bilinguis*; Figure 4.1, 4.2). We use spectral reflectances, toxicity assays, field abundance measurements, and predator learning experiments to investigate mechanisms that may be contributing to this pattern in nature.

Theoretical and empirical studies predict that coexistence of aposematic models may lead to (1) Batesian polymorphism (Ford 1971; Tuner 1987; Joron and Mallet 1998), (2) evolution of a mimic phenotype intermediate between model species (Edmunds 2000; Sherratt 2002)**,** or (3) mimetic resemblance to the most highly abundant and/or noxious model (Brower 1960, Goodale & Sneddon 1977; Lindström et al. 1997; Johnstone 2002). To test Batesian mimicry predictions, we quantify patterns of mimicry, abundance, and toxicity of models and mimic in the zone of overlap. We assessed mimicry by degree of overlap between model and mimic using 95% confidence ellipses computed from spectral reflectances (Endler 1990; Figure 4.1c). The mimic, *A. zaparo*, showed significant divergence in colour pattern across its geographic range predicted by colour differences between model species (Figure 4.1c). Where the two model species co-occur, however, the mimic's warning signal shows significant overlap with only *E. bilinguis* (Figure 4.1c). Thus, in contrast to predictions (1) and (2) for Batesian mimics sympatric with multiple models, *A. zaparo* is neither polymorphic nor intermediate.

Applying prediction (3) to this poison frog mimicry complex predicts that *A. zaparo* should mimic the more toxic and/or abundant model where *E. parvulus* and *E.*

bilinguis co-occur . To test this prediction, we measured relative abundance as encounter rate across an 8 km transect on 10 consecutive days near the Río Arajuno, Napo Province, Ecuador. We found *E. parvulus* to be more abundant (N = 43 in total; mean frogs per day ± SE; 4.3 ± 0.62) and *E. bilinguis* less abundant (N= 10 in total; 1.0 ± 0.26, Wilcoxon matched pairs test; Z = 2.716, $p_{2\text{-tail}} = 0.007$; Figure 4.2b). We assessed relative toxicity of the models and mimic using a standard protocol of frog skin-extract subcutaneous injection into laboratory mice (Daly & Myers 1967). Time to recovery from injection of *E. parvulus* skin-extract was significantly greater than the time to recovery from injection of *E. bilinguis* skin-extract (N = 5 mice per treatment, *E. parvulus* mean ± SE recovery time (minutes): 135.4 ± 9.31; *E. bilinguis*: 79.0 ± 3.19; Z = 2.023, $p_{2\text{-tail}} = 0.043$; Figure 4.2a). Injection of *A. zaparo* skin-extract caused no adverse reaction (no difference among reactions from *A. zaparo* skin-exact injections and saline control injections; *A. zaparo* = 5.2 ± 1.8; saline control 5.1 ± 1.3). Thus, in contrast to prediction (3), *A. zaparo* mimics the less abundant and less toxic model, *E. bilinguis*.

Mimics not only resemble the less toxic model species in the overlap zone, they also outnumber these models significantly (*A. zaparo* = 2.6/day ± 0.50; *E. bilinguis* = 1.0/day ± 0.26; $Z_{n=10} = 2.09$; $p_{2\text{-tail}} = 0.036$; Figure 4.2b,c). To investigate why mimicry of a less toxic and less abundant model might be favoured by selection, we conducted predator-learning experiments to explore the classical (Pavlov 1927; Duncan & Sheppard 1965) psychological phenomenon of 'stimulus generalization.' Naïve chicken predators were exposed to one of the model species in a series of learning trials, and then generalization of learned avoidance was assessed by subsequently exposing the educated

predator to the precise mimic phenotype (found in sympatry with the learning-stimulus) and the imperfect mimic phenotype (found in sympatry with the other model species). As predicted by single model studies (Duncan & Sheppard 1965; Goodale & Sneddon 1977; Lindström et al. 1997), we found that predator learning proceeded at a faster rate with the more toxic model, *E. parvulus* (*E. parvulus* mean learning slope = 40.33 ± 8.11; *E. bilinguis* mean learning slope = 18.04 ± 7.4; $Z_{n=6} = 1.992$, $p_{2\text{-tail}} = 0.046$). We tested mimic effectiveness (ability to deceive trained predators) and found that predators educated with either model (*E. bilinguis* and *E. parvulus*) generalize learned avoidance, on sight, to their respective mimic phenotype of *A. zaparo* (Figure 4.3; *E. bilinguis* mimic pre-learning time in quadrant (mean ± SE (seconds))= 25.83 ± 4.73; post-learning = 5.33 ± 1.05; *E. parvulus* mimic pre-learning time in quadrant= 25.83 ± 4.17; post-learning = 4.17 ± 1.54; $Z_{n=6} = 2.201$, $p_{2\text{-tail}} = 0.028$), providing the first empirical evidence for Batesian mimicry in dendrobatid frogs.

We further examined how broadly generalization of avoidance extends, or how imperfect a mimic can be and still gain protection from predators educated with a specific model. While precise mimics enjoyed equal protection regardless of the model species used for learning, imperfect mimics did not. Generalization of learned avoidance to the imperfect mimic differed depending on the toxicity of the model learning-stimulus (Figure 4.3; *E. parvulus* as learning-stimulus: mean ± SE post-learning time (seconds) with imperfect mimic = 6.67 ± 1.05; *E. bilinguis* as learning-stimulus: post-learning time with imperfect mimic = 26.67 ± 4.41; $Z_{n=6} = 2.207$; $p_{2\text{-tail}} = 0.027$). Predators educated

with the less toxic model, *E. bilinguis,* did not generalize learned avoidance to the mimic of *E. parvulus* (Figure 4.3a; baseline time in quadrant= 28.34 ± 4.41; post-learning time with imperfect mimic = 26.67 ± 4.41;$Z_{n=6}$ = 0.318; $p_{2\text{-tail}}$ = 0.75). In contrast, predators educated with the more toxic model, *E. parvulus,* did generalize learned avoidance to the imperfect mimic, the mimic of *E. bilinguis* (Figure 4.3b; baseline time = 49.17 ± 9.17; post-learning time with imperfect mimic = 6.67 ±1.05; $Z_{n=6}$ = 2.201; $p_{2\text{-tail}}$ = 0.028). Thus, the stimulus generalization gradient is broader when avoidance is learned on the more toxic model (avoidance generalizes to both mimic phenotypes), and, in contrast, the stimulus generalization gradient is more narrow when avoidance is learned on the less toxic model (avoidance generalizes to only the precise mimic phenotype; Figure 4.4; Duncan & Sheppard 1965; Sherratt 2002).

The relative selective advantage gained by either mimic phenotype in the zone of model species overlap is dependent on the penalty to the predator from the particular model being mimicked. Learned avoidance from experience with the more toxic model will generalize to either mimic phenotype; both mimic phenotypes receive protection if the predator has undergone avoidance learning with more toxic *E. parvulus* (Figure 4.4b). However, learned avoidance from experience with less toxic *E. bilinguis*, will only generalize to *E. bilinguis'* precise mimic (Figure 4.4a). Therefore, in the zone of model species overlap, mimics of *E. parvulus* only receive protection generated by *E. parvulus*, whereas mimics of *E. bilinguis* receive benefits generated by both models.

An alternative explanation for the apparent mimicry mismatch, wherein the mimic resembles the less toxic and less abundant model in the overlap zone, may be due to recent model range expansion (*E. parvulus*) or contraction (*E. bilinguis*) in this region. If the range of *E. parvulus* recently expanded north, or if *E. bilinguis* populations recently shrank in the overlap zone, then we may be capturing this species complex in an evolutionary lag snapshot—where the mimic (*A. zaparo*) has not had enough 'time' to show perfect mimicry to the more abundant and more toxic model. While no range transformation data is available to conclusively test this possibility, it does not rule out that toxicity-dependent generalized avoidance may maintain the current imbalance between mimic and model.

By mimicking the less toxic model (rather than mimetic polymorphism, an intermediate mimic phenotype, or mimicking the most toxic and/or numerous model) the increased predation risk accrued by an increased abundance of Batesian mimic individuals is spread over both defended model species, allowing the mimic to increase in abundance. This non-intuitive result is driven by toxicity-dependent generalization of learned avoidance; predators that learn on the more toxic model will generalize avoidance to the less toxic model's mimic, whereas predators that learn on the less toxic model show no generalization beyond this precise warning signal (Duncan & Sheppard 1965; Sherratt 2002). Thus, a mimic of the less toxic model can enjoy near complete protection from educated predators regardless of which model served for avoidance learning. We show strong evidence suggesting that the selective force influencing *A. zaparo*'s resemblance of the less toxic and less abundant model, *E. bilinguis*, is stimulus-controlled

predator generalization of learned avoidance. The present work therefore provides an adaptive hypothesis based on the classical psychological phenomenon of stimulus generalization (Pavlov 1927; Duncan & Sheppard 1965) which may help explain the paucity of Batesian polymorphism examples, and reveals a monomorphic evolutionary solution to the problem of Batesian abundance.

4.2 MATERIALS AND METHODS

Collection & Abundance Estimates. Fieldwork was conducted in Amazonian lowland rainforest, January–May 2003–2005. In February 2004, we measured poison frog encounter rates along ~8 km transect-trail for 10 consecutive days in models' overlap zone, Río Arajuno (~3 km SW of San Pedro), Napo, Ecuador. For reflectance measurements and predation experiments, we collected live frogs from: Estación Biológica Jatun Sacha, Napo (*Allobates zaparo* and *Epipedobates bilinguis*); Río Arajuno, Napo (*A. zaparo*, *E. bilinguis*, and *E. parvulus*); and Santiago, Morona-Santiago (*A. zaparo* and *E. parvulus*). For predation experiments, we collected brown, non-toxic *Colostethus awa* from western Ecuadorian cloudforest at Union del Tuachi, Pichincha. Taxonomy follows Frost (2004).

Colour analyses. Ninety-four frogs were collected and transported to Museo de Zoología, Universidad Católica del Ecuador (Figure 4.1c). Spectral reflectances were measured using an Ocean Optics PS2000 spectrometer, full spectrum light source (DT-

1000), Spectralon white standard, and reflectance probe (R400-7) at 2 mm distance from seven body-regions: head, dorsum, axilla, groin, vocal sac, flanks, and ventor (two measures per region). We collected leaf-litter background reflectances (Jatun Sacha: 6; Río Arajuno: 7; Santiago: 7). Forty-five habitat spectral irradiance measurements were collected at 0900 hrs on nine days with the PS2000 and cosine collector. Frog and background radiance estimates were computed as the product of spectral reflectances and average habitat irradiance spectrum.

To compare radiance measurements, independent of visual system, we employed Endler's (1990) segments classification method. Radiance spectra were divided into four bandwidths (UV: 300-399 nm, Short: 400-499; Middle: 500-599, and Long: 600-699), normalized by total intensity, and evaluated in a two-dimensional space by orthogonal axes representing hypothetical opponency processes (LS: Long – Short; MUV: Middle – UV). We computed composite Euclidean distances[20], D_{comp}, representing distance in colour space between frog and leaf-litter background. Whole-body colouration measures were similar between model species (*E. parvulus* $D_{comp} = 22.18 \pm 3.88$; *E. bilinguis* $D_{comp} = 20.24 \pm 5.30$; $t = 0.456$, $p_{2\text{-tail}} = 0.664$). To evaluate mimicry, we used multivariate discriminate functions analyses of warning coloured segments in JMP (Figure 4.1c; Sokal & Rohlf 1981, SAS Institute 2000).

Toxicity Assays. Five frogs from each species were euthanized and skinned following Darst et al. (2005). Methanol extracts from individual frogs were evaporated and resuspended in sterile saline. Resultant single-skin extracts were subcutaneously injected

into four treatments of five mice each (Daly & Myers 1967; N= 20 mice, IACUC #03110501): (1) *E. bilinguis*; (2) *E. parvulus*; (3) *A. zaparo*; or (4) saline-control injection. Sleeping behaviour was the baseline for toxicity assays. Mice were awakened with injection and time to complete recovery (return to sleep) was recorded. Recovery time was used to estimate degree of toxicity.

Predator learning experiments. While little data exists, birds may be potential poison frog predators (Master 1998). Thus, in Ecuador, we conducted a series of learning experiments using ~1 month old domestic chickens (*Gallus gallus domesticus*) as naïve, model predators (Osorio et al. 1999) and wild-caught dendrobatids (models: *E. bilinguis*, *E. parvulus*; mimic: *A. zaparo*). Birds were tested individually in a 1 m^2 dirt-floor test-arena of four 50 cm^2 quadrants outside, under natural lighting conditions. Chickens were fed chicken-mash and cracked-corn twice daily, and water *ad libitum*. We assessed mimic palatability by presenting six naïve chickens an *A. zaparo* (3 northern and 3 southern *A. zaparo*). Naïve chickens readily ate both *A. zaparo* and control frogs (*C. awa*).

We had two experimental groups (6 chicks each), differing in learning-stimulus (*E. parvulus* or *E. bilinguis*), in 8 learning trails (IACUC # 04071901). Learning trials consisted of presenting a chick with a learning-stimulus under a glass dome for 1 min or until the chick pecked the dome. The dome was then removed and latency to approach the stimulus was recorded up to 2 minutes or until a sampling event. In typical sampling events, chicks grab the frog in their beaks and spit the frog out. Only one chick ingested

a poison-frog (*E. bilinguis*), died three days later, and its data was removed. All other chicks tasted and released the frog; most frogs survived the sampling event. We defined learning rate as the slope (latency to peck / # of trials) until complete avoidance (no subsequent sampling in further trials). Control frogs were presented to chicks after trials #2 and #6 to assure chicks were still motivated to eat frogs.

After training was complete, learning and learning generalization were assessed in choice experiments which paired the control frog with one of three brightly coloured dendrobatids: toxic model learning-stimulus (learned avoidance); precise mimic of learning-stimulus (learning generalization); and imperfect mimic of learning-stimulus (degree of generalization). Chicks were presented with both the brightly coloured frog and control frog each under a glass dome for two minutes; time spent in each dome's test-arena quadrant was recorded. Frog placement within the test-arena was randomized across trials. We assessed learned avoidance and generalization of learned avoidance by comparing pre-learning (baseline) and post-learning time spent by chicks in the brightly coloured frog's test-arena.

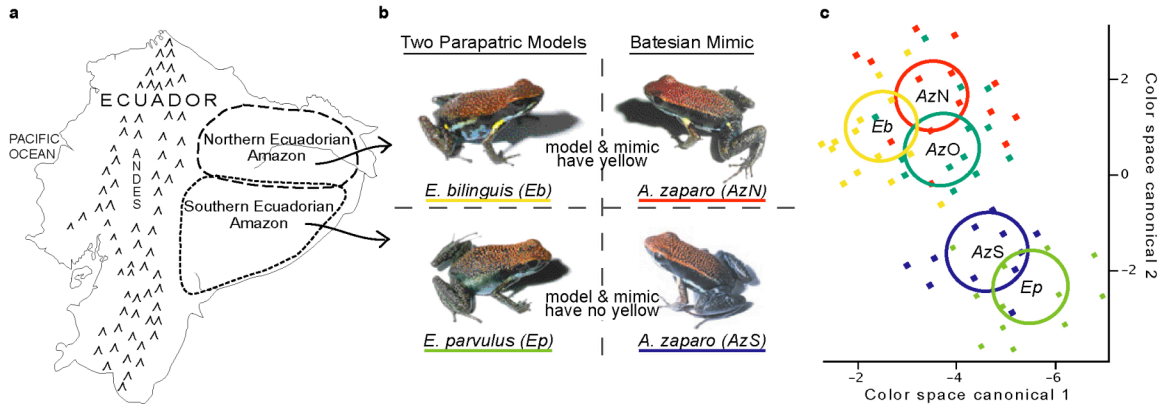

Figure 4.1. Poison frog mimicry complex and colour analyses. **a**, Geographic distribution of model and mimic species; **b**, model and mimic warning signals; **c**, discriminate functions plot: colour segments (radiance LS and MUV; Endler 1990) of 'aposematic' frog colours (red, yellow, and black) from individuals' head, dorsum, left and right axilla as covariates; species and locality as categories; mimicry was determined by overlap of model and mimic 95% confidence ellipses around the multivariate centroid (*Eb*: *E. bilinguis*, N = 25; *AzN*: *A. zaparo* sympatric with *E. bilinguis* in north, N = 15; *AzO*: *A. zaparo* from model species' zone of overlap, N = 13; *AzS*: *A. zaparo* sympatric with *E. parvulus* in south, N = 13; *Ep*: *E. parvulus*, N = 28).

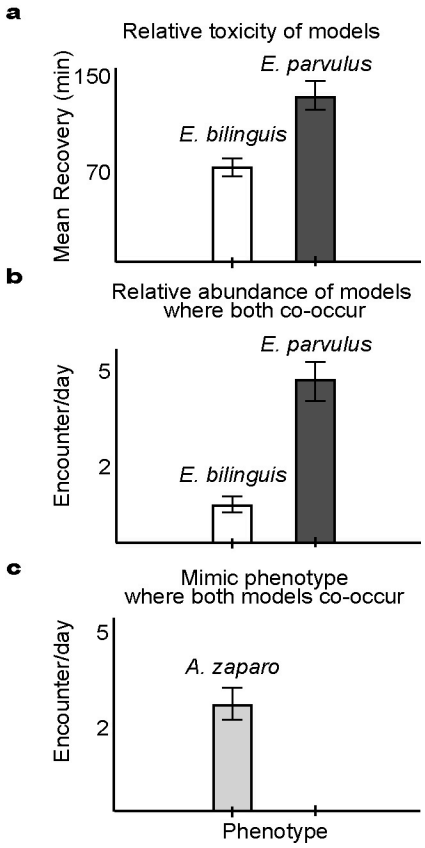

Figure 4.2. Measured features of poison frog model-mimic system (mean ± SE). **a**, Mice mean recovery time (minutes) post-injection with different model skin-extracts. Mean encounter rate per day for **b**, models, and **c**, mimic phenotype, where both models co-occur.

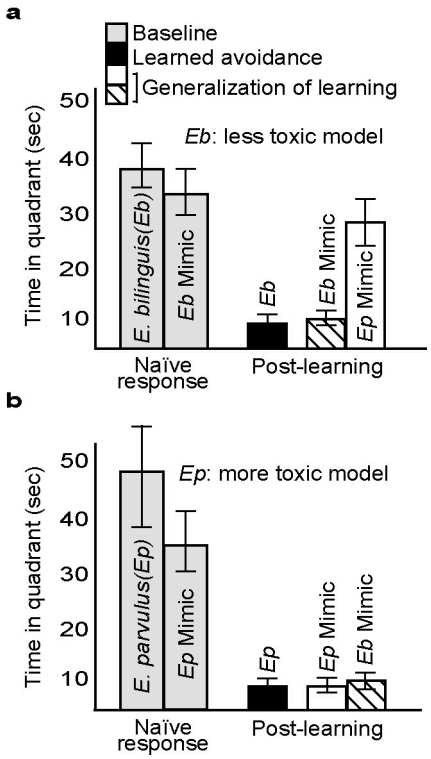

Figure 4.3. Predator avoidance learning: chicks' baseline vs. post-learning time (mean ± SE) in frog's test-quadrant. **a**, The less toxic model, *E. bilinguis*, as learning-stimulus; chicks learned to avoid *Eb*: *E. bilinguis* (baseline vs. post-learning time in frog's quadrant; $Z = -2.207$, $p_{2\text{-tail}} = 0.027$). Learned avoidance generalized to *Eb* mimic: *A. zaparo*-North ($Z = -2.201$, $p_{2\text{-tail}} = 0.028$), but avoidance did not generalize to *Ep* mimic: *A. zaparo*-South ($Z = -0.318$, $p_{2\text{-tail}} = 0.75$). **b**, The more toxic model, *E. parvulus*, as learning-stimulus; chicks learned to avoid *Ep*: *E. parvulus* ($Z = -2.201$, $p_{2\text{-tail}} = 0.028$). Learned avoidance generalized to *Ep* mimic: *A. zaparo*-South ($Z = -2.201$, $p_{2\text{-tail}} = 0.028$); avoidance also generalized to *Eb* mimic: *A. zaparo*-North ($Z = -2.207$, $p_{2\text{-tail}} = 0.027$).

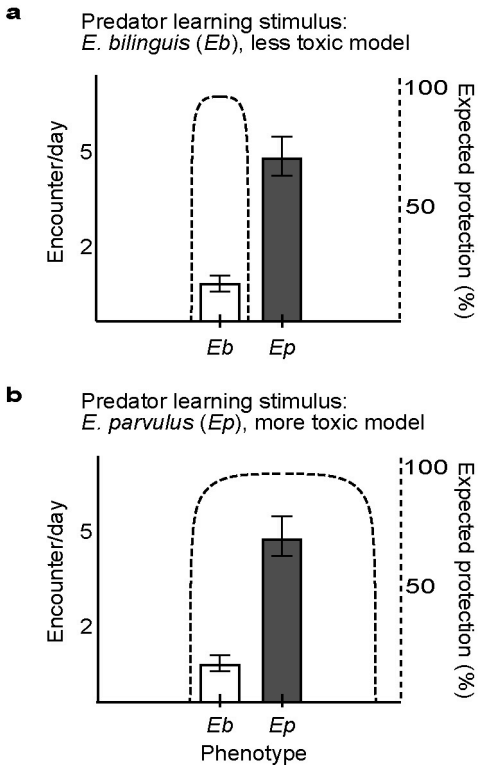

Figure 4.4. Generalized avoidance curves (dashed lines represent expected protection for each phenotype estimated using predator learning data presented in Figure 4.3). Estimates of protection assume fully trained predators in the wild. **a**, Less toxic model, *E. bilinguis*, as learning-stimulus: learned avoidance does not generalize beyond the warning signal with which predators were trained; **b**, more toxic model, *E. parvulus*, as learning-stimulus: learned avoidance generalizes to both *E. parvulus*' mimic and *E. bilinguis*' mimic. Thus, *A. zaparo* individuals resembling less toxic *E. bilinguis* gain a selective advantage no matter which model served as avoidance learning-stimulus.

References

Bates, H. W. 1862. Contributions to an insect fauna of the Amazon valley. Lepidoptera: Heliconidae. Transactions of the Linnean Society of London 23: 495–566.

Brodie, E. D., Jr. & Brodie, E. D. III 1980. Differential avoidance of mimetic salamanders by free-ranging birds. Science 208, 181–182.

Brower, J. V. Z. 1960. Experimental studies of mimicry. IV. The reactions of starlings to the different proportions of models and mimics. American Naturalist 94: 271–282.

Brower, L. P. & Brower, J. V. Z. 1962. The relative abundance of model and mimic butterflies in natural populations of the *Battus philenor* mimicry complex. Ecology 43: 154–158.

Daly, J. W. & Myers, C. W. 1967. Toxicity of Panamanian poison frogs (*Dendrobates*): some biological and chemical aspects. Science 156: 970–973.

Darst, C. R., Menéndez-Guerrero, P. A., Coloma, L. A., & Cannatella, D. C. 2005. Evolution of dietary specialization and chemical defense in poison frogs (Dendrobatidae): a comparative analysis. American Naturalist. 165: 56–69.

Duncan, C. J. & Sheppard, P. M. 1965. Sensory discrimination and its role in the evolution of Batesian mimicry. Behaviour 24: 269–282.

Edmunds, M. 1974. *Defence in Animals: A Survey of Anti-predator defences*. New York: Longman Group Limited; and references therein.

Edmunds, M. 2000. Why are there good mimics and poor mimics? Biological Journal of the Linnean Society 70: 459–466.

Endler, J. A. 1990. On the measurement and classification of colour in studies of animal colour patterns. Biological Journal of the Linnean Society 41: 315–352.

Fisher, R. A. 1930. *Genetical theory of natural selection* Oxford: Clarendon Press.

Ford, E. B. 1971. *Ecological Genetics*, 3rd Ed. London: Chapman and Hall Ltd.

Frost, D. R. 2004. Amphibian species of the world: an online reference, V 3.0. http://research.amnh.org/herpetology/amphibia/index.html; American Museum of Natural History, NY.

Goodale, M. A. & Sneddon, I. 1977. The effect of distastefulness of the model on the predation of artificial Batesian mimics. Animal Behaviour 25: 660–665.

Greene, H. W. & McDiarmid, R. W. 1981. Coral snake mimicry: does it occur? Science 213, 1207–1212.

Johnstone, R. A. 2002. The evolution of inaccurate mimics. Nature 418, 524-526.

Joron, M. & Mallet, J. L. B. 1998. Diversity in mimicry: paradox or paradigm? Trends in Ecology and Evolution 13: 461–466.

Lindström, L., Alatalo, R. V. & Mappes, J. 1997. Imperfect Batesian mimicry–the effects of the frequency and the distastefulness of the model. Proceedings of the Royal Society of London B 264: 149–153.

Master, T. L. 1998. *Dendrobates auratus* (black and green poison dart frog): Predation. Herpetological Review 29: 164–165.

Osorio, D., Vorobyev, M. & Jones, C. D. 1999. Colour vision of domestic chicks. Journal of Experimental Biology 202: 2951–2959.

Pavlov, I. P. 1927. *Conditioned reflexes* (translation, Anrep, G. V.) London: University Press.

Santos J. C., Coloma L. A., & Cannatella D. C. 2003. Multiple recurring origins of aposematism and diet specialization in poison frogs. Proceedings of the National Academy of Sciences USA 100: 12792–12797.

SAS Institute, Inc. 2000. JMP® Statistics and Graphics Guide, Version 4. SAS Institute Inc., Cary, North Carolina.

Sherratt, T. N. 2002. The evolution of imperfect mimicry. Behavioral Ecology 13: 821–826.

Sokal, R. R. & Rohlf, F. J. 1981. *Biometry: the principles and practice of statistics in biological research*. 2nd Ed. San Francisco: W.H. Freeman and Co.

Turner, J. R. G. 1987. The evolutionary dynamics of Batesian and Müllerian mimicry: similarities and differences. Ecological Entomology 12: 81–95; and references therein.

Wallace, A. R. 1865. On the phenomena of variation and geographical distribution as illustrated by the Papilionidae of the Malayan region. Transactions of the Linnean Society of London 25: 1-71.

Chapter 5

Predator learning, experimental psychology, and novel predictions for mimicry dynamics*

Abstract. The evolution of warning coloration and defensive mimicry is driven by predator avoidance learning and behaviour. Behavioural ecologists who study the evolution of warning signals have recognized the significance of predator learning and memory; however, the empirical work on animal learning from experimental psychology can further inform our predictions of predator behaviour. This paper explores how information from experimental psychology can improve our conceptualization of predator learning and memory. I consider how relevant findings in animal learning, particularly the importance of contextual cues in mediating behaviour, may provide new insight for the evolutionary and ecological dynamics of defensive mimicry. Specifically, work in animal learning psychology predicts that (1) a Batesian mimic will be less disadvantageous for the model species than previously assumed, (2) contextual cues will be a selective agent in behaviour and/or distribution of model and mimic individuals, and (3) multimodal signals will contribute to model and mimic species context specificity, particularly in species with no opportunity for unique ecological cues. These predictions may help explain ecological/behavioural empirical data currently considered incongruous with predictions from mimicry theory.

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5.1 INTRODUCTION

In the evolution of warning colouration and defensive mimicry, the predator learns from experience with the unpalatable, brightly coloured prey item to associate the warning signal with unpalatability. As a result of this learned association, the predator avoids prey with this bright colouration in future encounters, and learned avoidance generalizes to similarly coloured mimic species. As the selective agent, predator learning and behavior are an integral part of the evolution of warning colouration and mimetic convergence. The importance of receiver psychology in a system in which predator learning and behaviour are central has been widely recognized in behavioural ecology (Huheey 1976; Gittleman & Harvey 1980; Owen & Owen 1984; Turner et al. 1984; Leimar et al. 1986; Guilford 1990; Guilford & Dawkins 1991; Speed 1993; Turner & Speed 1996; MacDougall & Dawkins 1998; Yachi & Higashi 1998; Holmgren & Enquist 1999; Speed & Turner 1999; Speed 1999*a*, *b*, 2000; Servedio 2000; Speed et al. 2000; Mallet 2001; Speed 2001), and specific assumptions about predator learning and memory have greatly influenced predicted evolutionary outcomes for warning colouration and mimicry (Speed 1993; Tuner & Speed 1996; Speed 1999*b*; Servedio 2000; Speed 2001).

The extensive empirical research on animal learning and memory from experimental psychology, therefore, can further improve our conceptualization of predator behaviour and predictions for mimicry dynamics.

This paper explores how relevant information from experimental psychology can help inform behavioural ecology's approach to studying predator learning and memory. Such information suggests the behaviour-mediating contribution of contextual cues (i.e. cues other than those directly involved in simple associative learning) may provide new insights for the evolutionary and ecological dynamics of defensive mimicry (Bates 1862; Müller 1879). Evidence from animal learning psychology makes novel predictions for the evolution of warning colouration and mimicry, specifically that (1) an edible (Batesian) mimic may be less disadvantageous for the model than previously predicted, (2) contextual cues will be a selective agent in behaviour and/or distribution of model and mimic individuals, and (3) multimodal signals will contribute to model and mimic species context-specificity, particularly in species unable to exploit unique ecological contexts. These predictions may help to explain ecological/behavioural empirical evidence that appears inconsistent with current predictions of defensive mimicry.

5.2 BATESIAN MIMICRY AS COUNTERCONDITIONING

Most behavioural ecology modeling of predator avoidance learning makes use of a **classical conditioning** (bolded terms are defined in the Glossary 5.1) function based on

Bush & Mosteller (1951) and Rescorla & Wagner (1972). This view of predator avoidance learning interprets the experience with an edible mimic as reversing previous predator learning from experience with the brightly coloured, unpalatable prey (Huheey 1976; Owen & Owen 1984; Speed 1993; Turner & Speed 1996; Speed & Turner 1999; Speed 1999*a*, *b*). For example, experience with unpalatable, brightly coloured prey will lower the probability of future attack, whereas experience with a palatable mimic will raise the probability of future attack. In contrast, empirical work in animal learning psychology suggests that a successful predation encounter with the edible mimic is a **counterconditioning** trial (Speed 2000), the behavioural outcome of which is contextually controlled (Bouton 1994).

Counterconditioning occurs when the **conditioned stimulus** (CS: bright coloration) is experienced with a new **unconditioned stimulus** (new US: palatability rather than unpalatability), and results in interference (Bouton 1994). Specifically, counterconditioning results in **retroactive interference**, which can be thought of as subsequent learning of conflicting information; memory for something is disrupted by exposure to new information (Bouton 1993). Much research in experimental psychology has shown that retroactive interference does not result in the loss of the previously conditioned association; original learning is not destroyed (Bouton 2002 and refs therein). Rather, retroactive interference involves new learning that is stored along with the old learning, resulting in two available "meanings" for the CS, analogous to the properties of an ambiguous word (Bouton 2002). Such research indicates predator experience with a Batesian mimic will result in ambiguity in interpreting the warning signal, rather than the

loss of predator learning from previous experience with brightly coloured, unpalatable prey.

Evidence that original learning is not destroyed in counterconditioning is seen in phenomena such as **spontaneous recovery** (Bouton and Peck 1992), **renewal** (Peck and Bouton 1990) and **reinstatement** (Brooks et al. 1995). In all cases, the originally learned behaviour is recovered, showing that original learning is not lost after counterconditioning.

Spontaneous recovery. Spontaneous recovery is the recuperation of originally conditioned behaviour after a period of rest (Pavlov 1927). Empirical evidence suggests that counterconditioning performance is highly prone to spontaneous recovery; originally learned behaviour returns if the subject is tested after a delay following retroactive interference (Bouton & Peck 1992; Bouton 1993; Bouton et al. 1999). For example, if avoidance behaviour is counterconditioned by exposure to the mimic, spontaneous recovery of the originally acquired aversion after time, and the predator will avoid the brightly coloured prey item in spite of counterconditioning.

Renewal. Renewal is the recovery of originally learned behaviour when contextual cues present during retroactive interference are changed (Bouton & Bolles 1979). Work in experimental psychology has demonstrated that behaviour after retroactive interference is strongly determined by context, which includes all aspects of an encounter besides the CS and US (e.g. Bouton 1984; Bouton & Bolles 1979; Peck & Bouton 1990; Bouton 1993; Bouton & Brooks 1993). Experimental psychologists define all characteristics of the

testing environment as contextual cues, including physical features of the arena, lighting, smells, orientation, and ambient noise. Context is defined as such because, in simple associative conditioning, the association between the CS and US is what persists with change in testing environment. Features of the test subject's context appear to become discernibly important only after the CS acquires a level of ambiguity (such as in counterconditioning), requiring other cues to determine the meaning of the predictive stimulus.

When the original CS–US association (i.e. bright colouration–unpalatability association) is established in one context and interference (i.e. experience with a Batesian mimic) occurs in a second context, the original response to the CS is recovered when the animal is returned to the first context, as well as when the animal is moved to a neutral context (Bouton & Bolles 1979; Peck & Bouton 1990; Bouton & Brooks 1993; Bouton & Ricker 1994). For example, if the bright coloration–unpalatability association is learned in context A, and is counterconditioned by exposure to the mimic in context B, we will see the avoidance response, rather than counterconditioning behaviour, not only when the predator returns to context A, but in all contexts except context B. Conditioned responding generalizes well across contexts; whereas, a switch out of the exact context in which counterconditioning took place causes a loss of retroactive interference behaviour. Researchers have suggested that spontaneous recovery is simply a special case of the renewal effect; it involves a change in temporal context, which causes a renewal of originally conditioned behaviour (Bouton 1993; Brooks et al. 1995).

Reinstatement. Reinstatement is the recovery of originally conditioned behavior produced by exposure to the original US (Rescorla & Heth 1975). For example, after experience with an edible mimic and resultant retroactive interference of learned avoidance, an experience with unpalatability (regardless of the unpalatable prey item's colouration) will cause recuperation of the avoidance response to the warningly coloured prey. Empirical work on reinstatement suggests that context is important in recovery of responding (Bouton 1984; Bouton & Peck 1989; Brooks et al. 1995). When the experience with the US occurs in the context in which original conditioning took place, the reinstatement is strongest. Presentation of the US in the conditioning context appears to restore the excitatory properties of the CS associated with the US, illustrating that interference has little effect on the originally learned association (Brooks et al. 1995).

Spontaneous recovery, renewal, and reinstatement predict that we will see predator avoidance behaviour in spite of experience with a Batesian mimic without the predator having to re-learn the association between bright coloration and unpalatability. The predator's response to the warning signal after counterconditioning will become contextually-dependent, resulting in predator avoidance of brightly coloured prey more often than not. Such conservative behaviour may be selectively advantageous for the predator, particularly if the risk involved in an encounter with an unpalatable prey item is great. As a predator's inappropriate act of counterconditioned behaviour becomes more costly, it becomes more advantageous to be conservative, by usually avoiding brightly coloured prey. Cost, however, is not only a function of the risk involved in attacking unpalatable prey, it is also a function of the availability of alternative prey (Dill 1975;

Luedenman et al. 1981; Hetz & Slobodchikoff 1988; Kokko et al. 2003; Lindström et al. 2004). Fewer other food options, and resulting hunger, may drive a predator to take greater foraging risks than if alternative, non-brightly coloured prey were plentiful.

5.3 CONTEXT-DEPENDENT AVOIDANCE: PREDICTIONS FOR MIMICRY DYNAMICS

The natural world is full of possible contextual cues potentially analogous to experimental psychology's definition of context as "aspects of the training or testing other than the conditioned and unconditioned stimuli" (Glossary 5.1). Given this similarity and the importance of context in modulating learned behaviours, context certainly contributes to predator avoidance learning and behaviour. In the model-mimic system, contextual cues could include species' microhabitat differences, density/distribution differences, locomotor differences, olfactory cues, auditory cues, escape behaviour, and/or activity time differences. Visual predators' formation of search images (Bond & Kamil 2002), prioritization of information storage (Laughlin & Mendl 2004), as well as predators' sensible biases to cues most likely to predict the value of a prey item (Gamberale-Stille & Tullberg 2001), will probably influence which stimuli count as context.

The context-specificity of counterconditioned behaviour predicts that a Batesian mimic may be less disadvantageous for the model than previously thought (Edmunds

1974), because a switch out of the mimic's context, into any other context, will cause a recovery of the predator's originally conditioned avoidance response. Traditionally, the existence of a Batesian mimic is thought to be harmful to the model, because predator experience with the mimic should contribute to disassociating the bright colouration and unpalatability and result in increased predator attack of model individuals. Thus, the effectiveness of the warning signal wanes as the mimic becomes too abundant (Edmunds 1974). Frequency-dependent selection, therefore, traditionally predicts that the mimic will be scarce, resulting in a high model/mimic ratio. Phenomena such as spontaneous recovery, renewal, and reinstatement, however, predict that a switch out of the edible mimic's context, into any other context, will cause a recovery of avoidance behaviour, usually resulting in predator avoidance of brightly coloured prey (Table 5.1). Contextually-mediated avoidance behaviour may explain why actual model/mimic ratios have been lower than has been traditionally predicted (Table 5.1) (Owen 1970; Waldbauer & Sheldon 1971; Waldbauer et al. 1977).

The context dependency of learned behaviour, particularly after counterconditioning, predicts that contextual cues will be a selective agent in the distribution of model and Batesian mimic individuals (Table 5.1). Arnold (1978) hypothesized that a predator's performance should evolve in a manner that reflects the dispersion of unpalatable models and palatable mimics in the environment. Furthermore, memory should evolve so that "the predator remembers the model long enough to skip over clumps of models, but forgets soon enough so that it will sample (and thus benefit from) the palatable mimics that come in between" (p. 225; Bouton 1994). Evidence for

the context dependency of retroactive interference performance, however, predicts that contextual cues, rather than, or potentially in addition to, memory time, are selective agents in the distribution of model and mimic individuals. If the model is always encountered in a particular context and the mimic is always encountered in another, the predator will show counterconditioning performance in the mimic's context and learned avoidance in the model's context (Sherratt & Beatty 2003). For the model to remain protected, it should be distributed in one, predictable context that differs from the mimic's context. The edible mimic, however, is better protected when it occupies the model's context. As a result, a discrepancy in context between the model and mimic is beneficial to the model and harmful to the mimic; the mimic will evolve toward the model in its context, but the model will evolve away from the mimic in its context. These outcomes will be true not only for spatial context (i.e. micro-sympatry versus allopatry) but for other possible contextual cues as well, such as species' locomotor differences, olfactory cues, auditory cues, escape behaviour, and/or activity time differences. The same kind of evolutionary arms race has been suggested for the evolution of model and Batesian mimic colour pattern, in which selection will tend to favor mimics that are more similar to the model, but favour models that are distinct from edible mimics (Edmunds 1974).

Context dependency of predator behaviour after retroactive interference may help to explain the evolution of model/mimic specific behaviours and/or microhabitat associations (Table 5.1). For example, in their discussion of the diversity of model-mimic pairs which occupy particular stratifications of the Neotropical rainforest, Mallet & Joron

(1999 p. 224) state, “It would be hard to imagine birds ignoring butterflies a meter or two higher or lower than their normal flight height in the forest understory.” If flight height is an important contextual cue in the predator’s avoidance behaviour, however, then context may explain birds’ differential behaviour towards butterflies in different height microhabitats and may help to explain the evolution and maintenance of multiple mimicry rings. Tight association with a particular microhabitat or species-specific behaviour (Chai & Srygley 1990; Beccaloni 1997; DeVries & Lande 1999; Srygley 1999; Srygley & Ellington 1999; Golding et al. 2001) may contribute to species-specific contextual cues that could lead to recovery, renewal, and reinstatement of predator avoidance behaviour (Table 5.1). Those context-specific individuals will receive the selective advantage of recovered avoidance behaviour, contributing to the evolution of species-specific, predictable contexts.

Multimodal signals (i.e warning displays consisting of components in more than one sensory modality; Partan & Marler 1999), may also contribute to a species’ context specificity, particularly in species with no opportunity for unique ecological contextual cues (Table 5.1). Referring to odors and/or sounds emitted by brightly coloured insects when attacked by a predator, Rowe (2002) asks, “Given that conspicuous colour patterns are effective warning signals against avian predators, why have aposematic insects also evolved these additional signal components?” In domestic chicks, the addition of an odour to warning coloration produces stronger aversion than does colour alone, and the addition of sound improves speed of visual discrimination learning (Rowe & Guilford 1996; Rowe 1999; Rowe & Guilford 2001; Rowe 2002). Evidence from animal learning

psychology predicts that these kinds of multimodal, species-specific signals may also act as predictable contextual cues that elicit predator avoidance rather than counterconditioned behaviour. Loss of retroactive interference performance in the presence of contextual cues suggests another selective advantage of signaling in multiple sensory channels, providing further insight into the evolution of multimodal signals (Table 5.1) (Brower & Brower 1965; Rothschild & Haskell 1966; Rothschild et al. 1984).

5.4 CONCLUSIONS

Animal learning psychology can further inform our characterization of predator learning and memory in behavioural ecology. Contextual cues are important in mediating learned behaviour, particularly after a warning signal has acquired more than one meaning as a result of experience with a Batesian mimic (counterconditioning). Phenomena such as spontaneous recovery, renewal, and reinstatement will usually lead to the recuperation of originally learned avoidance, resulting in predator avoidance behaviour. The context-dependency of learned behaviour predicts that a Batesian mimic may be less disadvantageous for the unpalatable model than has been previously thought. Contextually mediated recovery of learned avoidance also predicts that context-specific prey will be afforded a selective advantage, contributing to the evolution of species-specific predictable cues, such as a tight association with a particular microhabitat, species-specific behaviour, and/or the use of multimodal signaling. These predictions

may help to explain some of mimicry's ecological/behavioural empirical evidence that appears incongruous with current predictions of mimicry theory (Table 5.1).

Table 5.1. Predictions for the evolution of warning colouration and mimicry based on data from experimental psychology.

Prediction	Expected outcome	Supporting ecological /behavioural empirical evidence
1) A Batesian mimic will not consistently elicit extra predator attacks on the model	Lower model/mimic ratio than previously predicted	Owen 1970; Waldbauer & Sheldon 1971; Waldbauer et al. 1977
2) Contextual cues will be selective agents in behavior and/or distribution of model/mimic individuals	Model/mimic specific behaviours and/or microhabitat associations	Chai & Srygley 1990; Beccaloni 1997; DeVries & Lande 1999; Srygley 1999; Srygley & Ellington 1999; Golding et al. 2001
3) Multimodal signals will contribute to context specificity in model/mimic species	Model/mimic specific multimodal signals will be observed, particularly in species with no opportunity for unique ecological contextual cues	Brower and Brower 1965; Rothschild & Haskell 1966; Rothschild et al. 1984

Glossary 5.1. Glossary of experimental psychology terms (bolded in text).

classical conditioning: a procedure in which a conditioned stimulus comes to be associated with an unconditioned stimulus

conditioned stimulus (CS): a stimulus that does not elicit a particular response initially, but comes to do so as a result of classical conditioning

context: all aspects of the training or testing other than the conditioned and unconditioned stimuli

counterconditioning: a procedure in which the originally conditioned stimulus is paired with a new unconditioned stimulus

reinstatement: recovery of originally learned behaviour produced by exposures to the unconditioned stimulus

renewal: recovery of originally learned behaviour produced by a shift away from the contextual cues present during retroactive interference

retroactive interference: disruption of memory caused by subsequent exposure to other information

spontaneous recovery: recovery of originally learned behaviour produced by a period of rest

unconditioned stimulus: a stimulus that elicits a particular response without the necessity of prior training

References

Arnold, S. J. 1978. The evolution of a special class of modifiable behaviors in relations to environmental pattern. American Naturalist 112: 415–427.

Bates, H. W. 1862. Contributions to an insect fauna of the Amazon valley. Lepidoptera: Heliconidae. Transactions of the Linnean Society of London 23: 495–566.

Beccaloni, G. 1997. Vertical stratification of ithomiine butterflies (Nymphalidae: Ithomiinae) mimicry complexes: the relationship between adult flight height and larval host plant. Biological Journal of the Linnaean Society 62: 313–341.

Bond, A. B. & Kamil, A. C. 2002. Visual predators select for crypticity and polymorphism in virtual prey. Nature 415: 609–613.

Bouton, M. E. 1984. Differential control by context in the inflation and reinstatement paradigms. Journal of Experimental Psychology: Animal Behavior Processes 10: 56–74.

Bouton, M. E. 1993. Context, time, and memory retrieval in the interference paradigms of Pavlovian learning. Psychological Bulletin 114: 80–99.

Bouton, M. E. 1994. Conditioning, remembering, and forgetting. Journal of Experimental Psychology: Animal Behavior Processes 20: 219–231.

Bouton, M. E. 2002. Context, ambiguity, and unlearning: sources of relapse after behavioral extinction. Biological Psychiatry 52: 976–986.

Bouton, M. E. & Bolles, R. C. 1979. Contextual control of the extinction of conditioned fear. Learning & Motivation 10: 445–466.

Bouton, M. E. & Brooks, D. C. 1993. Time and physical context effects in Pavlovian discrimination reversal. Journal of Experimental Psychology: Animal Behavior Processes 19: 165–179.

Bouton, M. E. & Peck, C. A. 1989. Context effects on conditioning, extinction, and reinstatement in an appetitive conditioning preparation. Animal Learning & Behavior 17: 188–198.

Bouton, M. E. & Peck, C. A. 1992. Spontaneous recovery in cross-motivational transfer (counterconditioning). Animal Learning & Behavior 20: 313–321.

Bouton, M. E. & Ricker, S. T. 1994. Renewal of extinguished responding in a second context. Animal Learning & Behavior 22: 317–324.

Bouton, M. E., Nelson, J. B., & Rosas, J. M. 1999. Stimulus generalization, context change, and forgetting. Psychological Bulletin 125: 171–186.

Brooks, D. C., Hale, B., Nelson, J. B., & Bouton, M. E. 1995. Reinstatement after counterconditioning. Animal Learning & Behavior 23: 383–390.

Brower, L. P., & Brower, J. V. Z. 1965. Experimental studies of mimicry. 8. Further investigations of honeybees (*Apis mellifera*) and their dronefly mimics (*Eristalis* spp.) American Naturalist 99: 173–187.

Bush, R. R., & Mosteller, F. 1951. A mathematical model for simple learning. Psychological Review 58: 313–323.

Chai, P. & Srygley, R. B. 1990. Predation and the flight, morphology, and temperature of Neotropical rain-forest butterflies. American Naturalist 135: 748–765.

DeVries, P. J., & Lande, R. 1999. Associations of co-mimetic ithomiine butterflies on small spatial and temporal scales in a neotropical rainforest. Biological Journal of the Linnaean Society 67: 73–85.

Dill, L. M. 1975. Calculated risk-taking by predators as a factor in Batesian mimicry. Cannadian Journal of Zoology 53: 1614–1621.

Edmunds, M. 1974. *Defence in Animals: A Survey of Anti-predator defences*. New York: Longman Group Limited.

Gamberale-Stille, G. & Tullberg, B. S. 2001. Fruit or aposematic insect? Context-dependent colour preferences in domestic chicks. Proceedings of the Royal Society of London B 268: 2525–2529.

Gittleman, J. L. & Harvey, P. H. 1980. Why are distasteful prey not cryptic? Nature 286: 149–150.

Golding, Y. C., Ennos, A. R., & Edmunds, M. 2001. Similarity in flight behaviour between the honeybee *Apis mellifera* (Hymenoptera: Apidae) and its presumed mimic,

the dronefly *Eristalis tenas* (Diptera: Syrphidae). Journal of Experimental Biology 204: 139–145.

Guilford, T. 1990. The evolution of aposematism. In*: Insect Defenses: Adaptive Mechanisms and Strategies of Prey and Predators*. (Ed. by D. L. Evans & J. O. Schmidt), pp. 23–61. New York: State University of New York Press.

Guilford, T., & Dawkins, M. S. 1991. Receiver psychology and the evolution of animal signals. Animal Behaviour 42: 1–14.

Hetz, M., & Slobodchikoff, C. N. 1988. Predation pressure on an imperfect Batesian mimicry complex in the presence of alternative prey. Oceologia 76: 570–573.

Holmgren, N. M. A., & Enquist M. 1999. Dynamics of mimicry evolution. Biological Journal of the Linnaean Society 66: 145–158.

Huheey, J. E. 1976. Studies on warning coloration and mimicry. IV. Evolutionary consequences of a Batesian-Müllerian spectrum; a model for Müllerian mimicry. Evolution 30: 86–93.

Kokko, H., Mappes, J., & Lindström, L. 2003. Alternative prey can change model-mimic dynamics between parasitism and mutualism. Ecology Letters 6: 1068–1076.

Laughlin, K. & Mendl, M. 2004. Cost of acquiring and forgetting information affect spatial memory and its susceptibility to interfere. Animal Behaviour 68: 97–103.

Leimar, O., Enquist, M., & Sillén-Tullberg, B. 1986. Evolutionary stability of aposematic coloration and prey unprofitability: a theoretical analysis. American Naturalist 128: 469–490.

Lindström, L. Alatalo, R. V., Lyytinen, A., & Mappes, J. 2004. The effect of alternative prey on the dynamics of imperfect Batesian and Müllerian mimicries. Evolution 58: 1294–1302.

Luedeman, J. K., McMorris, F. R., & Warner, D. D. 1981. Predators encountering a model-mimic system with alternative prey. American Naturalist 117: 1040–1048.

MacDougall, A. & Dawkins, M. S. 1998. Predator discrimination error and the benefits of Müllerian mimicry. Animal Behaviour 55: 1281–1288.

Mallet, J. 2001. Mimicry: an interface between psychology and evolution. Proceedings of the National Academy of Sciences USA 98: 8928–8930.

Mallet, J. & Joron, M. 1999. Evolution of diversity in warning color and mimicry: polymorphisms, shifting balance, and speciation. Annual Review of Ecology and Systematics 30: 201–233.

Müller, F. 1879. *Ituna* and *Thyridia*: a remarkable case of mimicry in butterflies. Transactions of the Entomological Society of London 1879: xx–xxix.

Owen, D. F. 1970. Mimetic polymorphism and the palatability spectrum. Okios 21: 333–336.

Owen, R. E. & Owen, A. R. G. 1984. Mathematical paradigms for mimicry: recurrent sampling. Journal of Theoretical Biology 109: 217–247.

Partan, S. & Marler, P. 1999. Communication goes multimodal. Science 283: 1272–1273.

Pavlov, I. P. 1927. Conditioned reflexes (G.V. Anrep, trans.) London: Oxford University Press.

Peck, C. A. & Bouton, M. E. 1990. Context and performance in aversive-to-appetitive and appetitive-to-aversive transfer. Learning & Motivation 21: 1–31.

Rescorla, R. A. & Heth C. D. 1975. Reinstatement of fear to an extinguished conditioned stimulus. Journal of Experimental Psychology: Animal Behavior Processes 1: 88–96.

Rescorla, R. A. & Wagner, A. R. 1972. A theory of Pavlovian conditioning: variations in the effectiveness of reinforcement and nonreinforcement. In*: Classical Conditioning II: Current Research and Theory*. (Ed. by A. H. Black & W. F. Prokasy), pp. 64–99. New York: Appleton-Century-Crofts.

Rothschild, M. & Haskell, P. T. 1966. Stridulation of the garden tiger moth *Arctia caja* L. audible to the human ear. Proceedings of the Royal Academy of Sciences London A41: 167–170.

Rothschild, M., Moore, B. P., & Brown, W. V. 1984. Pyrazines as warning odour components in the monarch butterfly, *Danaus plexippus*, and in moths of the genera

Zygaena and *Amata* (Lepidoptera). Biological Journal of the Linnean Society 23: 372–380.

Rowe, C. 1999. Receiver psychology and the evolution of multi-component signals. Animal Behaviour 58: 921–931.

Rowe, C. 2002. Sound improves visual discrimination learning in some avian predators. Proceedings of the Royal Society of London B 269: 1353–1357.

Rowe, C. & Guilford, T. 1996. Hidden colour aversions in domestic chicks triggered by pyrazine odours of insect warning displays. Nature 383: 520–522.

Rowe, C. & Guilford, T. 2001. The evolution of multimodal warning displays. Evolutionary Ecology 13: 655–671.

Servedio, M. R. 2000. The effects of predator learning, forgetting, and recognition errors on the evolution of warning coloration. Evolution 54: 751–763.

Sherratt, T. N. & Beatty, C. D. 2003. The evolution of warning signals as reliable indicators of prey defense. American Naturalist 162: 377–389.

Speed, M. P. 1993. Müllerian mimicry and the psychology of predation. Animal Behaviour 45: 571–580.

Speed, M. P. 1999a. Batesian, quasi-Batesian or Müllerian mimicry? Theory and data in mimicry research. Evolutionary Ecology 13: 755–776.

Speed, M. P. 1999b. Robot predators in virtual ecologies: the importance of memory in mimicry studies. Animal Behaviour 57: 203–213.

Speed, M. P. 2000. Warning signals, receiver psychology and predator memory. Animal Behaviour 60: 269–278.

Speed, M. P. 2001. Can receiver psychology explain the evolution of aposematism? Animal Behaviour 61: 205–216.

Speed, M. P. & Turner, J. R. G. 1999. Learning and memory in mimicry: II. Do we understand the mimicry spectrum? Biological Journal of the Linnean Society 67: 281–312.

Speed, M. P., Alderson, N. J., Hardman, C., & Ruxton, G. D. 2000. Testing Müllerian mimicry: an experiment with wild birds. Proceedings of the Royal Society of London B 267: 725–731.

Srygley, R. B. 1999. Locomotor mimicry in *Heliconius* butterflies: contrast analyses of flight morphology and kinematics. Philosophical Transactions of the Royal Society of London B 354: 203–214.

Srygley, R. B. & Ellington, C. P. 1999. Discrimination of flying mimetic, passion-vine butterflies *Heliconius*. Proceedings of the Royal Society of London B 266: 2137–2140.

Turner, J. R. G. & Speed, M. P. 1996. Learning and memory in mimicry. I. Simulations of laboratory experiments. Philosophical Transactions of the Royal Society of London B 351: 1157–1170.

Turner, J. R. G., Kearney, E. P., & Exton, L. S. 1984. Mimicry and the Monte Carlo predator: the palatability spectrum and the origins of mimicry. Biological Journal of the Linnean Society 23: 247–268.

Waldbauer, G. P. & Sheldon, J. K. 1971. Phenological relationships of some aculeate Hymenoptera, their dipteran mimics, and insectivorous birds. Evolution 25: 371–382.

Waldbauer, G. P., Sternburg, J. G., and Maier, C. T. 1977. Phenological relationships of wasps, bumblebees, their mimics, and insectivorous birds in an Illinois sand area. Ecology 58: 583–591.

Yachi, S. & Higashi, M. 1998. The evolution of warning signals. Nature 394: 882–884.

Consolidated References

Aliabadi, A., Renwick, J. A. A., & Whitman, D. W. 2002. Sequestration of glucosinolates by harlequin bug *Murgantia histrionica*. Journal of Chemical Ecology 28: 1749–1762.

Almendáriz, A. 1987. Contribucion al conocimiento de la herpetofauna centroiental ecuatoriana. Revista Politécnica, Quito 12: 144–177.

Alpin, R. T., Benn, M. H., & Rothschild, M. 1968. Poisonous alkaloids in the body tissues of the cinnabar moth (*Callimorpha jacobaeae* L.) Nature 219: 747–748.

Arnold, S. J. 1978. The evolution of a special class of modifiable behaviors in relations to environmental pattern. American Naturalist 112: 415–427.

Bates, H. W. 1862. Contributions to an insect fauna of the Amazon valley. Lepidoptera: Heliconidae. Transactions of the Linnean Society of London 23: 495–566.

Beccaloni, G. 1997. Vertical stratification of ithomiine butterflies (Nymphalidae: Ithomiinae) mimicry complexes: the relationship between adult flight height and larval host plant. Biological Journal of the Linnaean Society 62: 313–341.

Becerra, J. X. & Venable, D. L. 1999. Macro-evolution of insect-plant associations: the relevance of host biogeography to host affiliation. Proceedings of the National Academy of Sciences USA 96: 12626–12631.

Becerra, J. X. 1997. Insects on plants: macroevolutionary chemical trends in host use. Science 276: 253–256.

Berenbaum, M. 1983. Coumarins and caterpillars: a case for coevolution. Evolution 37: 163–179.

Bond, A. B. & Kamil, A. C. 2002. Visual predators select for crypticity and polymorphism in virtual prey. Nature 415: 609–613.

Bouton, M. E. & Bolles, R. C. 1979. Contextual control of the extinction of conditioned fear. Learning & Motivation 10: 445–466.

Bouton, M. E. & Brooks, D. C. 1993. Time and physical context effects in Pavlovian discrimination reversal. Journal of Experimental Psychology: Animal Behavior Processes 19: 165–179.

Bouton, M. E. & Peck, C. A. 1989. Context effects on conditioning, extinction, and reinstatement in an appetitive conditioning preparation. Animal Learning & Behavior 17: 188–198.

Bouton, M. E. & Peck, C. A. 1992. Spontaneous recovery in cross-motivational transfer (counterconditioning). Animal Learning & Behavior 20: 313–321.

Bouton, M. E. & Ricker, S. T. 1994. Renewal of extinguished responding in a second context. Animal Learning & Behavior 22: 317–324.

Bouton, M. E. 1984. Differential control by context in the inflation and reinstatement paradigms. Journal of Experimental Psychology: Animal Behavior Processes 10: 56–74.

Bouton, M. E. 1993. Context, time, and memory retrieval in the interference paradigms of Pavlovian learning. Psychological Bulletin 114: 80–99.

Bouton, M. E. 1994. Conditioning, remembering, and forgetting. Journal of Experimental Psychology: Animal Behavior Processes 20: 219–231.

Bouton, M. E. 2002. Context, ambiguity, and unlearning: sources of relapse after behavioral extinction. Biological Psychiatry 52: 976–986.

Bouton, M. E., Nelson, J. B., & Rosas, J. M. 1999. Stimulus generalization, context change, and forgetting. Psychological Bulletin 125: 171–186.

Braekman, J. C., Daloze, D., & Pasteels, J. M. 1982. Cyanogenic and other plant glucosides in a New Guinea bug *Leptocoris isolata*: possible precursors in its host plant. Biochemical Systematics and Ecology 10: 355–364.

Brodie, E. D., Jr. & Brodie, E. D. III 1980. Differential avoidance of mimetic salamanders by free-ranging birds. Science 208, 181–182.

Brooks, D. C., Hale, B., Nelson, J. B., & Bouton, M. E. 1995. Reinstatement after counterconditioning. Animal Learning & Behavior 23: 383–390.

Brower, J. V. Z. 1960. Experimental studies of mimicry. IV. The reactions of starlings to the different proportions of models and mimics. American Naturalist 94: 271–282.

Brower, L. P. 1958. Bird predation and food-plant specificity in closely related procryptic insects. American Naturalist 92: 183–187.

Brower, L. P. & Brower, J. V. Z. 1962. The relative abundance of model and mimic butterflies in natural populations of the *Battus philenor* mimicry complex. Ecology 43: 154–158.

Brower, L. P. & Brower, J. V. Z. 1964. Birds, butterflies and plant poisons: a study in ecological chemistry. Zoologica 49: 137–159.

Brower, L. P., & Brower, J. V. Z. 1965. Experimental studies of mimicry. 8. Further investigations of honeybees (Apis mellifera) and their dronefly mimics (Eristalis spp.) American Naturalist 99: 173–187.

Bush, R. R., & Mosteller, F. 1951. A mathematical model for simple learning. Psychological Review 58: 313–323.

Caldwell, J. P. 1996. The evolution of myrmecophagy and its correlates in poison frogs (family Dendrobatidae). Journal of Zoology, London 240: 75–101.

Chai, P. & Srygley, R. B. 1990. Predation and the flight, morphology, and temperature of Neotropical rain-forest butterflies. American Naturalist 135: 748–765.

Chiao, C.-C., Vorobyev, M., Cronin, T., Osorio, D. 2000. Spectral tuning of dichromats to natural scenes. Vision Research 40: 3257–3271.

Clough, M. & Summers, K. 2000. Phylogenetic systematics and biogeography of the poison frogs: evidence from mitochondrial DNA sequences. Biological Journal of the Linnean Society 70: 515–540.

Cott, H. B. 1940. Adaptive coloration in animals. London: Methuen.

Daly, J. W. 1998. Thirty years of discovering arthropod alkaloids in amphibian skin. Journal of Natural Products 61: 162–172.

Daly, J. W. 2003. Amphibian skin: a remarkable source of biologically active arthropod alkaloids. Journal of Medical Chemistry 46: 445–452.

Daly, J. W. & Myers, C. W. 1967. Toxicity of Panamanian poison frogs (*Dendrobates*): some biological and chemical aspects. Science 156: 970–973.

Daly, J. W., Karle, I., Myers, C. W., Tokuyama, T., Waters, J. A., & Witkop, B. 1971. Histrionicotoxins: Roentgen-ray analysis of the novel allenic and acetylenic spiroalkaloids isolated from a Colombian frog, *Dendrobates histrionicus*. Proceedings of the National Academy of Sciences USA 68: 1870–1875.

Daly, J. W., Myers, C. W., Warnick, J. E., & Albuquerque, E. X. 1980. Levels of batrachotoxin and lack of sensitivity to its action in poison-dart frogs (*Phyllobates*). Science 208: 1383–1385.

Daly, J. W., Myers, C. W., & Whittaker, N. 1987. Further classification of skin alkaloids from Neotropical poison frogs (Dendrobatidae), with a general survey of toxic noxious substances in the Amphibia. Toxicon 25: 1023–1095.

Daly, J. W., Garraffo, H. M. Spande, T. F., Jaramillo, C., & Rand, A. S. 1994. Dietary source for skin alkaloids of poison frogs (Dendrobatidae)? Journal of Chemical Ecology 20: 943–955.

Daly, J. W., Andriamaharavo, N. R., Andriantsiferana, M., & Myers, C. W. 1996. Madagascar poison frogs (*Mantella*) and their skin alkaloids. American Museum Novitates 3177: 1–34.

Daly, J. W., Garraffo, H. M., Hall, G. S. E., & Cover Jr., J. F. 1997. Absence of skin alkaloids in captive-raised Madagascan mantelline frogs (*Mantella*) and sequestration of dietary alkaloids. Toxicon 35: 1131–1135.

Daly, J. W., Garraffo, H. M., & Spande, T. F. 1999. Alkaloids from amphibian skin. Pages 1–161 *in* S. W. Pelletier, ed. Alkaloids: Chemical and Biological Perspectives (Vol. 13). Pergamon, New York.

Daly, J. W., Garraffo, H. M., Poonam, J., Spande, T. F., Snelling, R. R., Jaramillo, C., & Rand, A. S. 2000. Arthropod-frog connection: decahydroquinoline and pyrrolizidine alkaloids common to microsympatric myrmicine ants and dendrobatid frogs. Journal of Chemical Ecology 26: 73–85.

Daly, J. W., Garraffo, H. M., Spande, T. F., Clark, V. C., Ma, J., Ziffer, H., & Cover Jr., J. F. 2003. Evidence for an enantioselective pumiliotoxin 7-hydroxlase in dendrobatid poison frogs of the genus *Dendrobates*. Proceedings of the National Academy of Sciences USA 100: 11092–11097.

Daly, J. W., Kaneko, T., Wilham, J., Garraffo, H. M., Spande, T. F., Espinosa, A., Donnelly, & M. A. 2002. Bioactive alkaloids of frog skin: combinatorial bioprospecting reveals that pumiliotoxins have an arthropod source. Proceedings of the National Academy of Sciences USA 99: 13996–14001.

Darst, C. R. 2006. Predator learning, experimental psychology, and novel predictions for mimicry dynamics. Animal Behaviour, in press.

Darst, C R. & Cannatella, D. C. 2004. Novel relationships among hyloid frogs inferred from 12S and 16S mitochondrial DNA sequences. Molecular Phylogenetics and Evolution. 31: 462–475

Darst, C. R., & Cummings, M. E. 2006. Predator learning favours mimicry of a less toxic model in poison frogs. Nature. 404: 208–211.

Darst, C. R., Menéndez-Guerrero, P. A., Coloma, L. A., & Cannatella, D. C. 2005. Evolution of dietary specialization and chemical defense in poison frogs (Dendrobatidae): a comparative analysis. American Naturalist. 165: 56–69.

Darst, C. R., Cummings, M. E., & Cannatella, D. C. 2006. A mechanism for diversity in warning signals: conspicuousness versus toxicity in poison frogs. Proceedings of the National Academy of Sciences USA, in press.

Denno, R. F. S., Larsson, S., & Olmstead, K. L. 1990. Role of enemy-free space and plant quality in host-plant selection by willow beetles. Ecology 71: 124–137.

DeVries, P. J., & Lande, R. 1999. Associations of co-mimetic ithomiine butterflies on small spatial and temporal scales in a neotropical rainforest. Biological Journal of the Linnaean Society 67: 73–85.

Dill, L. M. 1975. Calculated risk-taking by predators as a factor in Batesian mimicry. Cannadian Journal of Zoology 53: 1614–1621.

Dobler, S. 2001. Evolutionary aspects of defense by recycled plant compounds in herbivorous insects. Basic Applied Ecology 2: 15–26.

Donnelly, M. A. 1991. Feeding patterns of the strawberry poison frog, *Dendrobates pumilio* (Anura: Dendrobatidae). Copeia 1991: 723–730.

Duellman, W. E. & Trueb, L. 1986. Biology of Amphibians. John Hopkins University Press, Baltimore, Maryland.

Duffey, S. S. 1980. Sequestration of plant natural products by insects. Annual Review of Entomology 25: 447–477.

Dumbacher, J. P., Beehler, J. W., Spande, T. F., Garraffo, H. M., & Daly, J. W. 1992. Homobatrachotoxin in the genus *Pitohui*: chemical defense in birds? Science 258: 799–801.

Dumbacher, J. P., Spande, T. F., & Daly, J. W. 2000. Batrachotoxin alkaloids from passerine birds: a second toxic bird genus (*Ifrita kowaldi*) from New Guinea. Proceedings of the National Academy of Sciences USA 97: 12970–12975.

Duncan, C. J. & Sheppard, P. M. 1965. Sensory discrimination and its role in the evolution of Batesian mimicry. Behaviour 24: 269–282.

Dunhan, A. E. 1983. Realized niche overlap: resource abundance and intensity of interspecific competition. Pages 261-280 *in* R. B. Huey, E. R. Pianka, and T. Shoener, eds. Lizard Ecology: Studies of a Model Organism. Harvard University Press, England.

Edmunds, M. 1966. Protective mechanisms in the Eolidacea (Mollusca: Nudibranchia). Journal of the Linnean Society (Zoology) 46: 27–71.

Edmunds, M. 1974. *Defence in Animals: A Survey of Anti-predator defences*. New York: Longman Group Limited.

Edmunds, M. 1987. Color in opisthobranchs. American Malacological Bulletin 5: 185–196.

Edmunds, M. 2000. Why are there good mimics and poor mimics? Biological Journal of the Linnean Society 70: 459–466.

Ehrlich, P. R. & Raven, P. H. 1964. Butterflies and plants: a study in coevolution. Evolution 18: 586–608.

Eisner, T. 1970. Chemical defense against predation in arthropods. Pages 157–217 *in* E. Sondheimer and J. B. Simeone, eds. Chemical Ecology. Academic Press, New York.

Eisner, T., Kafatos, F. C.,& Linsley, E. G. 1962. Lycid predation by mimetic adult Cerambycidae (Coleoptera). Evolution 16: 316–324.

Endler, J. A. 1990. On the measurement and classification of colour in studies of animal colour patterns. Biological Journal of the Linnean Society 41: 315–352.

Endler, J. A. & Mielke, P. W. 2005. Comparing entire colour patterns as birds see them. Biological Journal of the Linnean Society 86: 405–431.

Farrell, B. & Mitter, C. 1990. Phylogenies of insect/plant interactions: have Phyllobrotica leaf beetles (Chrysomelidae) and the Lamiales diversifies in parallel? Evolution 44: 1389–1403.

Feeny, P. P. 1987. The roles of plant chemistry in association between swallowtail butterflies and their host plants. Pages 53–61 *in* Proceedings of the 6th International Symposium on Insect-plant relations, Junk Publications, Dordrecht.

Felsenstein, J. 1985. Phylogenies and the comparative method. American Naturalist 125: 1–15.

Fisher, R. A. 1930. *Genetical theory of natural selection* Oxford: Clarendon Press.

Ford, E. B. 1971. Ecological Genetics, 3rd Ed. London: Chapman and Hall Ltd.

Freckleton, R. P, Harvey, P. H. & Pagel, M. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. American Naturalist 160: 712–726.

Fritz, G., Rand, A. S., & dePamphilis, C. W. 1981. The aposematically colored frog, *Dendrobates pumilio*, is distasteful to the large, predatory ant, *Paraponera clavata*. Biotropica 13: 158–159.

Frost, D. R. 2002. Amphibian species of the world: an online reference. V2.21. Electronic database available at research.amnh.org/herpetology/amphibia/index.html; American Museum of Natural History, NY.

Frost, D. R. 2004. Amphibian species of the world: an online reference, V 3.0. http://research.amnh.org/herpetology/amphibia/index.html; American Museum of Natural History, NY.

Futuyma, D. J. 1983. Evolutionary interactions among herbivorous insects and plants. Pages 207–231 *in* D. J. Futuyma and M. Slatkin, eds. Patterns and Processes in the Evolution of Life. Springer-Verlag, Berlin.

Gamberale-Stille, G. & Tullberg, B. S. 2001. Fruit or aposematic insect? Context-dependent colour preferences in domestic chicks. Proceedings of the Royal Society of London B 268: 2525–2529.

Garland, T. Jr., Harvey, P. H., & Ives, A. R. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. Systematic Biology 41: 18–32.

Garland, T. Jr., Huey, R. B., & Bennett, A. F. 1991. Phylogeny and thermal physiology in lizards: a reanalysis. Evolution 45: 1969–1975.

Garraffo, H. M., Caeres, J., Daly, J. W., Spande, T. F., Andriamaharavo, N. R. & Andriantsiferana, M. 1993a. Alkaloids in Madagascan frogs (*Mantella*): pumiliotoxins,

indolizidines, quinolizidines, and pyrrolizidines. Journal of Natural Products 56: 1016–1038.

Garraffo, H. M., Spande, T. F., Daly, J. W., Baldessari, A., & Gros, E. G. 1993b. Alkaloids from bufonid toads (*Melanophryniscus*): decahydroquinolines, pumiliotoxins, indolizidines, pyrrolizidines, and quinolizidines. Journal of Natural Products 56: 357–373.

Gittleman, J. L. & Harvey, P. H. 1980. Why are distasteful prey not cryptic? Nature 286: 149–150.

Golding, Y. C., Ennos, A. R., & Edmunds, M. 2001. Similarity in flight behaviour between the honeybee *Apis mellifera* (Hymenoptera: Apidae) and its presumed mimic, the dronefly *Eristalis tenas* (Diptera: Syrphidae). Journal of Experimental Biology 204: 139–145.

Goodale, M. A. & Sneddon, I. 1977. The effect of distastefulness of the model on the predation of artificial Batesian mimics. Animal Behaviour 25: 660–665.

Gould, S. J, & Lewontin, R. C. 1979. The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationists programme. Proceedings of the Royal Society of London B 205: 581–598.

Greene, H. W. & McDiarmid, R. W. 1981. Coral snake mimicry: does it occur? Science 213, 1207–1212.

Guilford, T. 1990. The evolution of aposematism. In*: Insect Defenses: Adaptive Mechanisms and Strategies of Prey and Predators*. (Ed. by D. L. Evans & J. O. Schmidt), pp. 23–61. New York: State University of New York Press.

Guilford, T., & Dawkins, M. S. 1991. Receiver psychology and the evolution of animal signals. Animal Behaviour 42: 1–14.

Hart, N. S., Partridge, J. C., Cuthill, I. C. 1998. Visual pigments, oil droplets and cone photoreceptor distribution in the European starling (*Sturnus vulgaris*) Journal Experimental Biology 201: 1433–1446.

Harvey, P. H., & Pagel, M. D. 1991. The comparative method and evolutionary biology. Oxford University Press, Oxford, England.

Hetz, M., & Slobodchikoff, C. N. 1988. Predation pressure on an imperfect Batesian mimicry complex in the presence of alternative prey. Oceologia 76: 570–573.

Holmgren, N. M. A., & Enquist M. 1999. Dynamics of mimicry evolution. Biological Journal of the Linnaean Society 66: 145–158.

Huheey, J. E. 1976. Studies on warning coloration and mimicry. IV. Evolutionary consequences of a Batesian-Müllerian spectrum; a model for Müllerian mimicry. Evolution 30: 86–93.

Ivlev, V.S. 1961. Experimental ecology of the feeding of fishes (Transl. by D. Scott). Yale University Press, New Haven.

Jacobs, J. 1974. Quantitative measurement of food selection: a modification of the forage ratio and Ivlev's electivity index. Oceologia 14: 413–417.

Jeffries, M. J. & Lawton, J. 1984. Enemy free space and the structure of ecological communities. Biological Journal of the Linnaean Society 23: 269–286.

Johnstone, R. A. 2002. The evolution of inaccurate mimics. Nature 418, 524-526.

Jones, T. H., Gorman, J. S. T., Snelling, R. R., Delabie, J. H. C., Blum, M. S., Garraffo, H. M., Jain, P., Daly, J. W., & Spande, T. F. 1999. Further alkaloids common to ants and frogs: decahydroquinolines and a quinolizidine. Journal of Chemical Ecology 25: 1179–1193.

Joron, M. & Mallet, J. L. B. 1998. Diversity in mimicry: paradox or paradigm? Trends in Ecology and Evolution 13: 461–466.

Kokko, H., Mappes, J., & Lindström, L. 2003. Alternative prey can change model-mimic dynamics between parasitism and mutualism. Ecology Letters 6: 1068–1076.

La Marca, E. 1992. Catálogo taxonómico, biogeográfica y bibliográfico de las ranas de Venezuela. Mérida, Venezuela. Cuaderno Geográfico de Universidad de Los Andes 9: 1–197.

La Marca, E. 1994. Taxonomy of the frogs of the genus *Mannophryne* (Amphibia: Anura: Dendrobatidae). Publicaciones de la Asociación de Amigos de Doñana 4: 1-75.

Laughlin, K. & Mendl, M. 2004. Cost of acquiring and forgetting information affect spatial memory and its susceptibility to interfere. Animal Behaviour 68: 97–103.

Leimar, O., Enquist, M., & Sillén-Tullberg, B. 1986. Evolutionary stability of aposematic coloration and prey unprofitability: a theoretical analysis. American Naturalist 128: 469–490.

Lindström, L., Alatalo, R. V. & Mappes, J. 1997. Imperfect Batesian mimicry–the effects of the frequency and the distastefulness of the model. Proceedings of the Royal Society of London B 264: 149–153.

Lindström, L., Alatalo, R. V., Mappes, J., Riipi, M., Vertainen, L. 1999. Can aposematic signals evolve by gradual change? Nature 397: 249–251.

Lindström, L. Alatalo, R. V., Lyytinen, A., & Mappes, J. 2004. The effect of alternative prey on the dynamics of imperfect Batesian and Müllerian mimicries. Evolution 58: 1294–1302.

Luedeman, J. K., McMorris, F. R., & Warner, D. D. 1981. Predators encountering a model-mimic system with alternative prey. American Naturalist 117: 1040–1048.

Lüling, K. H. 1971. Der Fäberfrosch *Phyllobates bicolor* Bibron der Cordillera Azul (Peru). Bonner Zoologische Beitrage 22: 161–174.

MacDougall, A. & Dawkins, M. S. 1998. Predator discrimination error and the benefits of Müllerian mimicry. Animal Behaviour 55: 1281–1288.

Maddison, W. P. 1990. A method for testing the correlation of two binary characters: Are gains and losses concentrated on certain branches of a phylogenetic tree? Evolution 44: 539–557.

Mallet, J. & Joron, M. 1999. Evolution of diversity in warning color and mimicry: polymorphisms, shifting balance, and speciation. Annual Review of Ecology and Systematics 30: 201–233.

Mallet, J. 2001. Mimicry: an interface between psychology and evolution. Proceedings of the National Academy of Sciences USA 98: 8928–8930.

Mappes, J., Marples, N., & Endler, J. A. 2005. The complex business of aposematism. Trends in Ecology and Evolution 20: 598–603.

Martins, E. P. & Hansen, T. F. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. American Naturalist 149: 646–667.

Master, T. L. 1998. *Dendrobates auratus* (black and green poison dart frog): Predation. Herpetological Review 29: 164–165.

Mebs, D. 2001. Toxicity in animals: trends in evolution? Toxicon 39: 87–96.

Müller, F. 1879. *Ituna* and *Thyridia*: a remarkable case of mimicry in butterflies. Transactions of the Entomological Society of London 1879: xx–xxix.

Myers, C. W. 1987. New generic names for some Neotropical poison frogs (Dendrobatidae). Papéis Avulsos de Zoologia de São Paulo 36: 301–306.

Myers, C. W. & Daly, J. W. 1976. Preliminary evaluation of skin toxins and vocalizations in taxonomic and evolutionary studies of poison-dart frogs (Dendrobatidae). Bulletin of the American Museum of Natural History 157: 174–262.

Myers, C. W., Daly, J. W. & Malkin, B. 1978. A dangerously toxic new frog (*Phyllobates*) used by the Embera Indians of Western Colombia, with discussion of blowgun fabrication and dart poisoning. Bulletin of the American Museum of Natural History 161: 307–365.

Myers, C. W., Paolillo O., A., & Daly, J. W. 1991. Discovery of a defensively malodorous and nocturnal frog in the family Dendrobatidae: phylogenetic significance of a new genus and species from the Venezuelan Andes. American Museum Novitates 3002: 1–33.

Neter, J., Kutner, M. H., Nachtsheim, C. J., & Wasserman, W. 1996. Applied Linear Regression Models. Third Edition. R. D. Irwin, Illinois.

Neuwirth, M., Daly, J. W., Myers, C. W., & Tice, L.W. 1979. Morphology of the granular secretory glands in skin of poison-dart frogs (Dendrobatidae). Tissue & Cell 11: 755–771.

Nishida, R. 2002. Sequestration of defensive substances from plants by Lepidoptera. Annual Review of Entomology 47: 57–92.

Orians, G. H. & Paine, R. T. 1983. Convergent evolution at the community level, Pages 431–458, *in* D. J. Futuyma and M. Slatkin, eds., Coevolution. Sinauer, Sunderland, Massachusetts.

Osorio, D., Vorobyev, M. & Jones, C. D. 1999. Colour vision of domestic chicks. Journal of Experimental Biology 202: 2951–2959.

Owen, D. F. 1970. Mimetic polymorphism and the palatability spectrum. Okios 21: 333–336.

Owen, R. E. & Owen, A. R. G. 1984. Mathematical paradigms for mimicry: recurrent sampling. Journal of Theoretical Biology 109: 217–247.

Pagel, M. 1993. Seeking the evolutionary regression coefficient: an analysis of what comparative methods measure. Journal of Theoretical Biology 164: 191–205.

Pagel, M. 1997. Inferring evolutionary processes from phylogenies. Zoologica Scripta 26: 331–348.

Pagel, M. 1999. Inferring the historical patterns of biological evolution. Nature 401: 877–884.

Parmelee, J. R. 1999. Trophic ecology of a tropical anuran assemblage. Scientific Papers of the Natural History Museum, University of Kansas 11: 1-59.

Partan, S. & Marler, P. 1999. Communication goes multimodal. Science 283: 1272–1273.

Pavlov, I. P. 1927. *Conditioned reflexes* (translation, Anrep, G. V.) London: University Press.

Peck, C. A. & Bouton, M. E. 1990. Context and performance in aversive-to-appetitive and appetitive-to-aversive transfer. Learning & Motivation 21: 1–31.

Pianka, E. R. 1986. Ecology and Natural History of Desert Lizards: Analyses of the Ecological Niche and Community Structure. Princeton University Press, Princeton, New Jersey.

Poulton, E. B. 1890. The Colours of Animals: Their Meaning and Use, Especially Considered in the Case of Insects. Kegan Paul, Trench, Trübner, London (International Scientific Series 68).

Purvis, A. & Rambaut, A. 1994. Comparative analysis by independent contrasts (CAIC). Version 2. Department of Zoology, Oxford University, Oxford.

Rescorla, R. A. & Heth C. D. 1975. Reinstatement of fear to an extinguished conditioned stimulus. Journal of Experimental Psychology: Animal Behavior Processes 1: 88–96.

Rescorla, R. A. & Wagner, A. R. 1972. A theory of Pavlovian conditioning: variations in the effectiveness of reinforcement and nonreinforcement. In*: Classical Conditioning II: Current Research and Theory*. (Ed. by A. H. Black & W. F. Prokasy), pp. 64–99. New York: Appleton-Century-Crofts.

Roper, T. J., & Redston, S. 1987. Conspicuousness of distasteful prey affects the strength and durability of one-trial avoidance learning. Animal Behaviour 35: 739–747.

Rothschild, M. & Haskell, P. T. 1966. Stridulation of the garden tiger moth Arctia caja L. audible to the human ear. Proceedings of the Royal Academy of Sciences London A41: 167–170.

Rothschild, M., Moore, B. P., & Brown, W. V. 1984. Pyrazines as warning odour components in the monarch butterfly, Danaus plexippus, and in moths of the genera *Zygaena* and *Amata* (Lepidoptera). Biological Journal of the Linnean Society 23: 372–380.

Rowe, C. 1999. Receiver psychology and the evolution of multi-component signals. Animal Behaviour 58: 921–931.

Rowe, C. 2002. Sound improves visual discrimination learning in some avian predators. Proceedings of the Royal Society of London B 269: 1353–1357.

Rowe, C. & Guilford, T. 1996. Hidden colour aversions in domestic chicks triggered by pyrazine odours of insect warning displays. Nature 383: 520–522.

Rowe, C. & Guilford, T. 2001. The evolution of multimodal warning displays. Evolutionary Ecology 13: 655–671.

Rowell-Rahier, M. 1984. The food plant preferences of *Phratora vitellinae* (Coleoptera: Chrysomelidae) A. Field observations. Oecologia 64: 369–374.

Ruxton, G. D., Sherratt, T. N., & Speed, M. P. 2004. *Avoiding attack: the evolutionary ecology of crypsis, aposematism, and mimicry*. Oxford: Oxford University Press.

Santos J. C., Coloma L. A., & Cannatella D. C. 2003. Multiple recurring origins of aposematism and diet specialization in poison frogs. Proceedings of the National Academy of Sciences USA 100: 12792–12797.

Saporito, R. A., Donnelly, M. A., Hoffman, R. L., Garraffo, H. M., & Daly, J. W. 2003. A siphonotid millipede (*Rhinotus*) as the source of spiropyrrolizidine oximes of dendrobatid frogs. Journal of Chemical Ecology 29: 2781–2786.

Saporito, R.A., Garraffo, H.M., Donnelly, M.A., Edwards, A.L., Longino, J.T., & Daly, J.W. 2004. Formicine ants: an arthropod source for the pumiliotoxin alkaloids of dendrobatid poison frogs. Proceedings of the National Academy of Sciences USA 101:8045–8050.

SAS Institute, Inc. 2000. JMP® Statistics and Graphics Guide, Version 4. SAS Institute Inc., Cary, North Carolina.

Schaffner, U., Boevé, J.-L., Gfeller, H., & Schlunegger, U. P. 1994. Sequestration of *Veratrum* alkaloids by specialist *Rhadinoceraea nodicornis* konow (Hymenoptera, Tenthredinidae) and its ecoethological implications. Journal of Chemical Ecology 20: 3233–3250.

Scudder, G. G. E., & Duffey, S. S. 1972. Cardiac glycosides in the Lygaeinae. Canadian Journal of Zoology 50: 35–52.

Servedio, M. R. 2000. The effects of predator learning, forgetting, and recognition errors on the evolution of warning coloration. Evolution 54: 751–763.

Sherratt, T. N. 2002. The evolution of imperfect mimicry. Behavioral Ecology 13: 821–826.

Sherratt, T. N. & Beatty, C. D. 2003. The evolution of warning signals as reliable indicators of prey defense. American Naturalist 162: 377–389.

Sherratt, T. N., Speed, M. P., & Ruxton, G. D. 2004. Natural selection on unpalatable species imposed by state-dependent foraging behaviour. Journal of Theoretical Biology 228: 217–226.

Siddiqi, A., Cronin, T. W., Loew, E. R., Vorobyev, M., & Summers, K. 2004. Interspecific and intraspecific views of color signals in the strawberry poison frog *Dendrobates pumilio*. Journal of Experimental Biology 207: 2471–2485.

Sillén-Tullberg, B. 1993. The effect of biased inclusion of taxa on the correlation between discrete characters in phylogenetic trees. Evolution 47: 1182–1191.

Simon, M. P. & Toft, C. A. 1991. Diet specialization in small vertebrates: mite-eating in frogs. Oikos 67: 483–489.

Simpson, E. H. 1949. Measurement of diversity. Nature 163: 688.

Sokal, R. R. & Rohlf, F. J. 1981. *Biometry: the principles and practice of statistics in biological research*. 2nd Ed. San Francisco: W.H. Freeman and Co.

Spande, T. F., Garraffo, H. M., Edwards, M. W., Yeh, H. J. C., Pannell, L., & Daly, J. W. 1992. Epibatidine: a novel (chloropyridyl) azabicycloheptane with analgesic activity from an Ecuadorian poison frog. Journal of the American Chemistry Society 114: 3475–3478.

Spande, T. F., Jain, P., Garraffo, H. M., Pannell, L. K., Yeh, H. J. C., Daly, J. W., Fukumoto, S., Imamura, K., Tokuyama, T., Torres, J. A., Snelling, R. R., & Jones, T. H. 1999. Occurrence and significance of decahydroquinolines from dendrobatid poison frogs and a myrmicine ant: use of 1H and 13C NMR and their conformational analysis. Journal of Natural Products 62: 5–21.

Speed, M. P. 1993. Müllerian mimicry and the psychology of predation. Animal Behaviour 45: 571–580.

Speed, M. P. 1999a. Batesian, quasi-Batesian or Müllerian mimicry? Theory and data in mimicry research. Evolutionary Ecology 13: 755–776.

Speed, M. P. 1999b. Robot predators in virtual ecologies: the importance of memory in mimicry studies. Animal Behaviour 57: 203–213.

Speed, M. P. 2000. Warning signals, receiver psychology and predator memory. Animal Behaviour 60: 269–278.

Speed, M. P. 2001. Can receiver psychology explain the evolution of aposematism? Animal Behaviour 61: 205–216.

Speed, M. P. & Turner, J. R. G. 1999. Learning and memory in mimicry: II. Do we understand the mimicry spectrum? Biological Journal of the Linnean Society 67: 281–312.

Speed, M. P, & Ruxton, G. D. 2005a. Warning displays in spiny animals: one (more) evolutionary route to aposematism. Evolution 59: 2499–2508.

Speed, M. P, & Ruxton, G. D. 2005b. Aposematism: what should our starting point be? Proceedings of the Royal Society of London B: 272: 431–438.

Speed, M. P., Alderson, N. J., Hardman, C., & Ruxton, G. D. 2000. Testing Müllerian mimicry: an experiment with wild birds. Proceedings of the Royal Society of London B 267: 725–731.

Srygley, R. B. 1999. Locomotor mimicry in *Heliconius* butterflies: contrast analyses of flight morphology and kinematics. Philosophical Transactions of the Royal Society of London B 354: 203–214.

Srygley, R. B. & Ellington, C. P. 1999. Discrimination of flying mimetic, passion-vine butterflies *Heliconius*. Proceedings of the Royal Society of London B 266: 2137–2140.

Strong, D. R. 1979. Biogeographic dynamics of insect-host plant communities. Annual Review of Entomology 24: 89–120.

Summers, K. 1999. *Dendrobates auratus* (green poison frog): Predation. Herpetolgoical Review 30: 91.

Summers, K. & Clough, M. E. 2001. The evolution of coloration and toxicity in the poison frog family (Dendrobatidae). Proceedings of the National Academy of Sciences USA 98: 6227–6232.

Summers, K. 2003. Convergent evolution of bright coloration and toxicity in frogs. Proceedings of the National Academy of Sciences USA 100: 12533–12534.

Szelistowski, W. A. 1985. Unpalatability of the poison arrow frog *Dendrobates pumilio* to the ctenid spider *Cupiennius coccineus*. Biotropica 17: 345–346.

Termonia, A., Pasteels, J. M., Windsor, D. M., & Milinkovitch, M. C. 2002. Dual chemical sequestration: a key mechanism in transitions among ecological specialization. Proceedings of the Royal Society of London B 269: 1–6.

Thompson, T. E. 1960. Defensive adaptations in opisthobranchs. Journal of the Marine Biological Association of the United Kingdom 39: 123–134.

Toft, C. A. 1980. Feeding ecology of thirteen syntopic species of anurans in a seasonal tropical environment. Oecologia 45: 131–141.

Toft, C. A. 1981. Feeding ecology of Panamanian litter anurans: patterns in diet and foraging mode. Journal of Herpetology 15: 139–144.

Toft, C. A. 1995. Evolution of diet specialization in poison-dart frogs (Dendrobatidae). Herpetologica 51: 202–216.

Turner, J. R. G. 1987. The evolutionary dynamics of Batesian and Müllerian mimicry: similarities and differences. Ecological Entomology 12: 81–95.

Turner, J. R. G. & Speed, M. P. 1996. Learning and memory in mimicry. I. Simulations of laboratory experiments. Philosophical Transactions of the Royal Society of London B 351: 1157–1170.

Turner, J. R. G., Kearney, E. P., & Exton, L. S. 1984. Mimicry and the Monte Carlo predator: the palatability spectrum and the origins of mimicry. Biological Journal of the Linnean Society 23: 247–268.

Vences, M., Glaw, F., & Böhme, W. 1997/98. Evolutionary correlates of microphagy in alkaloid-containing frogs (Amphibia: Anura). Zoologischer Anzeiger 236: 217–230.

Vences, M., Kosuch, J., Boistel, R., Haddad, C. F., La Marca, E., Lötters, S., & Veith, M. 2003. Convergent evolution of aposematic coloration in Neotropical poison frogs: a molecular phylogenetic perspective. Organismal Diversity and Evolution 3: 215–226.

von Euw, J., Fishelson, J., Parsons, J. A. , & Reichstein, T. 1967. Cardenolides (heart poisons) in a grasshopper feeding on milkweeds. Nature 214: 3–39.

Vorobyev, M., Osorio, D., Bennett, A. T. D., Marshall, N. J., & Cuthill, C. 1998. Tetrachromy, oil droplets and bird plumage colors. Journal of Comparative Physiology A 183: 621–633.

Waldbauer, G. P. & Sheldon, J. K. 1971. Phenological relationships of some aculeate Hymenoptera, their dipteran mimics, and insectivorous birds. Evolution 25: 371–382.

Waldbauer, G. P., Sternburg, J. G., & Maier, C. T. 1977. Phenological relationships of wasps, bumblebees, their mimics, and insectivorous birds in an Illinois sand area. Ecology 58: 583–591.

Wallace, A. R. 1867. *Proceedings of the Entomological Soceity of London*, 4 March, pp IXXX–IXXXi.

Wallace, A. R. 1889. *Darwinism—an exposition of the theory of natural selection with some applications*. London: Macmillian.

Yachi, S. & Higashi, M. 1998. The evolution of warning signals. Nature 394: 882–884.

Vita

Catherine Rachel Darst was born in Salinas, California on January 23, 1978, the daughter of Deirdre and Philip Darst. After completing her secondary education at Santa Catalina School in Monterey, California, in 1996, she attended University of California, Davis. She graduated in June 2001 with honors from UC Davis with a Bachelor's of Arts and Sciences (B.A.S) in Evolution & Ecology and English (double major). She entered the Ecology, Evolution, & Behavior graduate program at the University of Texas, Austin, in September 2001.

Permanent address: 23085 Guidotti Place, Salinas, CA 93908

This dissertation was typed by the author.